Volume 16 MARCH-APRIL, 1951 Number 2

Food Research

An Official Publication of

The Institute of Food Technologists

Heureux déjà d'avoir pu servir mes concitoyens et l'humanite, je me repose sur la justice, la générosité et les lumières d'un gouvernement sagé qui ne cesse d'encourager et de protéger toutes les découvertes utiles.

—NICOLAS APPERT.

Published Six Times a Year in February, April, June, August, October, and December

Food Research

An Official Publication of
The Institute of Food Technologists

Volume 16 MARCH-APRIL, 1951 Number 2

EDITORIAL BOARD

Z. I. KERTESZ, *Editor-in-Chief*
New York State Agricultural Experiment Station
Geneva, New York

E. J. CAMERON	L. B. JENSEN	CATHERINE J. PERSONIUS
EVELYN G. HALLIDAY	M. A. JOSLYN	R. W. PILCHER
ROBERT S. HARRIS	MAX LEVINE	SAMUEL C. PRESCOTT
L. B. HOWARD	A. G. LOCHHEAD	J. J. WILLAMAN

FRED W. TANNER, *Editor Emeritus*

Institute of Food Technologists Committee on Publications

WALTER D. PHETEPLACE, *Chairman*

PHILIP K. BATES	CARL R. FELLERS
JOHN T. KNOWLES	GEORGE F. STEWART

PAUL F. SHARP, *(Ex Officio)*

CHARLES S. LAWRENCE, *Business Manager*
176 West Adams Street
Chicago 3, Illinois

Published by the Institute of Food Technologists six times a year. Printed by The Garrard Press, 119-123 West Park Avenue, Champaign, Illinois. Entered as second-class matter at the Post Office at Champaign, Illinois, under the act of March 3, 1879.

Special Note: First published in 1947, this review of enrichment requirements in the U.S.A. is now brought up to date by the inclusion of milled white rice, the latest grain food to be fortified with vitamins and minerals. This year, the Tenth Anniversary of white bread and flour enrichment, is an appropriate time to publish a new edition of this popular, helpful resume. We shall be pleased to supply extra copies gratis. Just write our Vitamin Division.

The ROCHE REVIEW *of enrichment requirements*

ALL FIGURES REPRESENT MILLIGRAMS PER POUND

PRODUCT	Thiamine (B_1)		Riboflavin (B_2)		Niacin		Iron	
	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.
Enriched BREAD, or other baked products	1.1	1.8	0.7	1.6	10.0	15.0	8.0	12.5
Enriched FLOUR[1]	2.0	2.5	1.2	1.5	16.0	20.0	13.0	16.5
Enriched FARINA[2]	1.66	–	1.2	–	6.0	–	6.0	–
Enriched MACARONI products[3]	4.0	5.0	1.7	2.2	27.0	34.0	13.0	16.5
Enriched NOODLE products[3]	4.0	5.0	1.7	2.2	27.0	34.0	13.0	16.5
Enriched CORN MEALS	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched CORN GRITS[4]	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched MILLED WHITE RICE[5]	2.0	–	*		16.0	–	13.0	–

1. *In enriched self-rising flour, calcium is also required between limits of 500-1500 mg. per pound.* 2. *No maximum levels have been enforced.* 3. *Levels allow for 30-50% losses in kitchen procedure.* 4. *Levels must not fall below 85% of minimum figures after a specific rinsing test described in the Federal Standards of Identity.* 5. *Levels must not fall below 85% of levels shown after washing and rinsing.*

**Omitted in the U.S.A. and Puerto Rico but used in certain Far Eastern countries to a minimum level of 1.2 mg. per pound.*

The maximum and minimum levels shown above for enriched bread, enriched flour, enriched farina, enriched macaroni, spaghetti and noodle products, enriched corn meal and corn grits are in accordance with Federal Standards of Identity or State laws. Act No. 183 of the Government of Puerto Rico requires the use of enriched flour for all products made wholly or in part of flour. This includes crackers, pretzels, etc.

The levels for milled white rice are officially those of the Government of Puerto Rico. These levels are commonly accepted in U. S. domestic and export marketing and are based on the recommendation of the Committee on Cereals, Food and Nutrition Board, National Research Council.

ROCHE **VITAMINS FOR ENRICHMENT**

VITAMIN DIVISION · HOFFMANN-LA ROCHE INC. · NUTLEY 10, N. J.

In Canada: Hoffmann-La Roche, Ltd., Montreal, Que.

Volume 16 MARCH-APRIL, 1951 Number 2

CONTENTS

STUDIES ON THE POSSIBILITY OF THE TRANSMISSION OF SALMONELLA BY COOKED FOWL. Dorothy L. Hussemann and Margaret A. Wallace 89

THE GROWTH INITIATION OF BACTERIA IN DEFROSTED EGGS. S. E. Hartsell . . . 97

SOME EFFECTS OF PROCESSING ON THE NUTRITIVE PROPERTIES OF PROTEINS. R. R. Baldwin, J. R. Lowry, and Reinhardt Thiessen, Jr. 107

GROWTH OF BACTERIA ASSOCIATED WITH FOOD POISONING EXPERIMENTALLY INOCULATED INTO DEHYDRATED MEAT. Milton Segalove and G. M. Dack 118

A STUDY OF METHODS OF PREPARING FOOD PRODUCTS FOR MICROBIOLOGICAL ANALYSES. A. H. Jones and W. E. Ferguson 126

THE NUMBERS AND TYPES OF BACTERIA FOUND ON THE HANDS OF FOOD HANDLERS. Murray P. Horwood and Virgil A. Minch 133

CHICKEN FLAVOR: THE FRACTIONATION OF THE VOLATILE CONSTITUENTS. Robert J. Bouthilet 137

CONSTITUTION OF THE JUICE LIPIDS OF THE FLORIDA VALENCIA ORANGE (*Citrus sinensis* L.). Lyle James Swift and M. K. Veldhuis 142

QUALITY OF ARTIFICIALLY RIPENED TOMATOES FOR CANNING PURPOSES. John Skok 147

THE EFFECT OF CERTAIN PREFREEZING TREATMENTS ON THE QUALITY OF EIGHT VARIETIES OF CULTIVATED HIGHBUSH BLUEBERRIES. Mary L. Greenwood, Martha Potgieter, and C. I. Bliss 154

NUTRITIONAL VALUES OF PROTEINS IN VARIOUS CEREAL BREAKFAST FOODS. Barnett Sure 161

EFFECT OF CRYSTAL SIZE ON SALT PENETRATION OF PORK LOINS. F. H. Smith, T. N. Blumer, Alexa C. Williams, and W. E. Tyler 166

SUBSCRIPTION RATES

To members of the Institute of Food Technologists, $4.50 per volume.

Non-members: Domestic, $5.50 per volume; foreign, $6.00 per volume.

Subscriptions and renewals are entered to begin with the first issue of the current volume.

Single copies of current volume: Domestic, $1.00; foreign, $1.15.

CORRESPONDENCE

Editorial: Z. I. Kertesz, Editor-in-Chief, New York State Agricultural Experiment Station, Geneva, New York.

Non-Member Subscriptions, Back Volumes, Single Copies, and Reprints: FOOD RESEARCH, 119-123 W. Park Ave., Champaign, Ill.

Members Subscriptions: THE INSTITUTE OF FOOD TECHNOLOGISTS, 176 W. Adams St., Chicago 3, Ill.

Advertising: Charles S. Lawrence, Business Manager, 176 W. Adams St., Chicago, Ill.

CLAIMS

Claims for copies lost in the mails must be received within 30 days (90 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

NOTE

The Institute of Food Technologists assumes no responsibility for statements in articles appearing in FOOD RESEARCH. The opinions expressed do not not necessarily reflect policies of the Institute of Food Technologists.

Manuscripts for Food Research should conform to the style used in the journal and be submitted in duplicate to the Editor-in-Chief. Authors of papers intended for FOOD RESEARCH should request from the Editorial Office a copy of the booklet "Suggestions to Authors." FOOD RESEARCH reserves the privilege of editing manuscripts to make them conform with the adopted style of the journal or returning them to the author for revision. Changes made by the Editorial Office may be reviewed by authors when they examine proof.

Copyright, 1951
By the Institute of Food Technologists
Printed in U. S. A.

YOUR *Indispensable* INSTRUMENT

. . . if you make any of these 28 products

- ACETIC ACID
- ALCOHOL
- BERRY PRESERVES
- BEVERAGES
- BOROSILICATE CROWN GLASS
- BROMONAPHTHALENE
- BUTYRIC ACID
- CHEESE PRODUCTS
- DISTILLED WATER
- EGG SOLIDS
- ETHYLANTRACENE
- EXTRA DENSE FLINT
- FLINT GLASS
- FLUORINATED HYDROCARBONS
- FRUIT BUTTERS
- HONEY
- HYDROGENATED FATS
- IODINE NUMBER (FLAXSEED, SOYBEAN)
- JELLIES, JAMS FRUIT PRESERVES
- MAPLE SYRUP
- NAPHTHALENE
- NYLON
- OIL CONTENT
- PAPER MILLS (STARCH SOLIDS)
- PENTANE
- PLASTICS
- TOMATO PRODUCTS
- WAXES (PARAFFIN, CARNAUBA)

Be sure it's *the world's finest* Abbe-type refractometer—the B&L "Abbe-56"!

Exclusive B&L advantages save time, cut costs, increase operator efficiency and comfort . . . in industrial quality control, in research, and in experimentation. Quick, positive determination of the percentage of dissolved solids in foods. Highest-accuracy identification and testing of transparent and opaque liquids and solids, by either transmitted or reflected light. Here are a few of the "Abbe-56" features you'll appreciate in *your* work.

SPARE PRISM SET—Quickly, easily interchanged; ends "down" time. (Optional)

FIXED-POSITION EYEPIECES—Mounted close together and inclined, for speed and comfort.

INTERNAL GLASS SCALE—Protected from dirt, corrosion. Index, 1.30 to 1.71; theoretical accuracy, ±.0001.

PROTECTIVE HOUSING—Streamlined metal, safeguards scale and moving parts.

WRITE for complete information and a demonstration to Bausch & Lomb Optical Company, 765-4 St. Paul St., Rochester 2, N. Y.

Bausch & Lomb "*Abbe-56*" Refractometer

STUDIES ON THE POSSIBILITY OF THE TRANSMISSION OF SALMONELLA BY COOKED FOWL[a,b,c]

DOROTHY L. HUSSEMANN AND MARGARET A. WALLACE[d]
Department of Home Economics, University of Wisconsin, Madison, Wisconsin

[Received for publication, May 29, 1950]

That the occurrence of Salmonella food poisoning is something of a present day public health problem is attested to by the fact that the Federal Public Health Service reported approximately 770 cases of such illness as occurring in the United States during 1948. Fowl and eggs frequently have been suggested as possible vehicles for conveying this type of food-borne infection to man. The question arises as to the effectiveness of common cookery procedures in protecting people from illness, when foods, especially fowl or eggs, infected with Salmonella are consumed. The work here described had as its objective the ascertaining of whether certain types of Salmonella which may be harbored in fowl muscle or liver were destroyed during cookery.

The relationship of the temperatures attained during cookery to the destruction of Salmonella present in eggs has been quite widely investigated. Rettger, Hull, and Sturges (1916) reported the effect of common cookery procedures on Salmonella present in whole fresh eggs which had been artificially contaminated. Other papers followed including that of Verder and Sutton (1933) who studied cookery temperatures of custards as related to survival of *Salmonella enteritidis*, of Cathcart, Merz, and Ryberg (1942) who observed the possibility of survival of Salmonella following cookery of starch-thickened custards and custard pies, of Gibbons and Moore (1944) who examined Salmonella-contaminated scrambled eggs, omelets, custards, sponge cakes, and muffins subsequent to cookery, and of Solowey and Calesnick (1948) who determined the effect of the temperatures encountered in scrambling eggs on Salmonella-infected reconstituted egg powders.

There appears to be less evidence available relative to the effect of cookery on Salmonella which may be harbored in fowl flesh. The work of Cherry, Scherago, and Weaver (1943) demonstrated that viable Salmonella were found to exist in 5.2 per cent of 250 samples of meat and poultry collected from retail meat markets in the region of Lexington, Kentucky. Thus, it would appear that Salmonella may continue to live in muscle or glandular tissue after the death of the host animal. A second

---

[a] Presented before the Tenth Annual Meeting of the I.F.T., Chicago, Illinois, May 23, 1950.

[b] Published with the permission of the Director of the Agricultural Experiment Station, Madison, Wisconsin.

[c] This study was financed by a grant from the Wisconsin Alumni Research Foundation.

[d] Present address: School of Home Economics, Michigan State College, East Lansing, Michigan.

point of consideration is the fact that Salmonella-infected fowl cannot as a rule be detected by their physical appearance, Jungherr (1940); Beister and Devries (1943). Hence, it would seem possible to have such birds move in commercial channels. Furthermore, fowl, especially turkey and chicken, have frequently been mentioned as the suspected source of infection when Salmonella food poisoning outbreaks occur. Among others Mosher, Wheeler, and Hardy (1941) and Myers (1946) have reported such observations. One further consideration seems pertinent. An analysis of the more recent studies which have been made on the serological types of Salmonella recovered from man [Borman *et al.* (1943); Edwards and Bruner (1943); Bornstein (1943)] and from fowl [Edwards (1939); Edwards, Bruner, and Morow (1948); Hinshaw, McNeil, and Taylor (1944)] indicates a great overlapping of types from these two sources.

For several reasons, then, it appeared that the presence in fowl muscle or liver of viable Salmonella which could cause food poisoning in man might be a distinct possibility and that it would be appropriate to initiate work which would seek to determine whether common methods of cookery could be relied upon to destroy these organisms. In undertaking such a study evidence was gathered from two types of endeavor. First, the survival rate of Salmonella in chickens proven to be infected with approximately known numbers of viable cells was determined subsequent to controlled broiling or roasting procedures. Secondly, samples of cooked poultry served in public eating establishments were collected and examined for the presence of viable Salmonella.

EXPERIMENTAL PROCEDURE

In that section of the work in which an attempt was made to determine the survival rate of Salmonella in fowl muscle or liver subsequent to controlled cooking methods, New Hampshire chickens were used. When the chickens were received in the laboratory they were pullorum tested by the method of Bunyea (1946). Since none of the chickens were found to be pullorum positive, all were housed, two to a cage, and given an adequate ration.

The experimental procedure for each individual bird included the following steps:

(a) One ml. of blood was drawn from the brachial vein of the right wing and diluted 1:10 with sterile saline. Using tryptose agar and SS agar, pour plates were made from the blood for the purpose of establishing whether any microorganisms were present prior to the laboratory-induced infection. (This step was omitted in some cases.)

(b) A 0.5-ml. suspension representing approximately 1.45×10^9 viable cells of *Salmonella typhimurium* 84 or a 1-ml. suspension representing approximately 3.2×10^8 viable cells of *Salmonella pullorum*, A-66, was introduced into the left brachial vein of the fowl. The lesser amount was injected into the broilers and the greater into the roasting chickens. This inoculum was prepared by suspending Salmonella cells grown on tryptose agar (Difco) slants for 24 hours at 37°C. in sterile saline, and adjusting to a turbidity permitting a 12 per cent light transmission on a photoelectric colorimeter.

(c) One hour was allowed to elapse and then 1-ml. blood sample was withdrawn from the left brachial vein. This blood was diluted and plated on SS agar in order to establish the number of viable Salmonella circulating in the blood stream.

(d) The chicken was killed immediately by decapitation and dressed and drawn by home methods.

(e) Raw tissue samples for bacteriological analysis were taken from the right side of the bird. The sites of sampling included the breast muscle, the heavy inside

musculature of the leg, the lower back muscle near the tail, and the liver. The samples from the leg and breast were taken with sterile number 6 or number 7 cork borers and those from the tail and liver were cut with sterile scissors. Each of these samples was weighed by difference in a sterile weighing bottle and incubated 24 hours at 37°C. in tetrathionate enrichment broth (Difco).

(f) The left side of the broiling chickens and the entire roasting chickens were cooked by methods currently advised by home economists for home use. Since in this laboratory some difficulty was encountered in correlating an appropriate degree of doneness of the meat and a specific temperature of either breast or leg muscle, the degree of cooking for the chickens was judged by timing. The total time of cookery for the broilers was 41 minutes; for the roasters 35 minutes per pound was used. The rate of heating was followed by placing copper-constantan thermocouples in the breast and thigh of the broiling chickens. These positions were analogous to those from which the samples were drawn. Similarly, thermocouples were placed in the breast, leg, and tail region of the roasting chickens. A thermometer was used in the liver samples. The temperature of the broiling oven and the oven also were determined by a thermocouple. Temperature readings were made every 3 minutes for the broilers and every 5 minutes for the roasting chickens, except for the liver for which temperature was determined at the end of the broiling period or at 15-minute intervals for the roasting chickens.

The broiling chickens were placed on a rack one-half inch above the bottom of a shallow, uncovered pan in a preheated electric oven adjusted to broil. The pan was placed 11 inches below the broiling unit. The chickens were broiled with the cavity side up for 20 minutes, then turned and broiled for an average of 21 minutes. The liver was placed on the broiler rack when the chicken was turned. The larger chickens were roasted, breast down, on a rack one-half inch above the bottom of a shallow uncovered pan in an electric oven, with the regulator set at 325°F.(163°C.). The liver was placed in the oven during the last hour of roasting.

(g) Immediately following removal of the chicken from the broiling oven or the oven, a series of samples was taken from the same positions on the left side of the bird as had been utilized in sampling the right side of the raw bird. The samples obtained were treated in a manner identical with the raw samples.

(h) To prepare both the raw and cooked samples for bacteriological analysis, each was ground on a power mixer in the tetrathionate broth in which it had been incubated. Qualitative tests for the presence of viable Salmonella were made by using SS agar and preparing duplicate streak plates of the original ground sample. Quantitative tests were made on this same medium by preparing triplicate pour plates of various dilutions of the original ground samples. All plates were incubated for 24 hours at 37°C. and then examined. Counts were made of the pour plates. Representative colonies from both types of plates were picked and transferred to Krumweide's triple sugar agar slants. After a 24-hour incubation, smears of those cultures which produced a yellow butt with gas and a red slope were Gram-stained. Transfers of those cultures demonstrated to be short, Gram-negative, non-sporforming rods were sent to the Salmonella Typing Station at Lansing, Michigan for positive identification.

(i) It seemed that the possibility of cross contaminating foods with Salmonella organisms after handling diseased fowl might exist. To test this premise the worker, after drawing and washing the roasting hens dried her hands on a clean towel and sliced liver sausage with a sterile knife. The liver sausage was left at room temperature in a sterile Petri dish. Samples of liver sausage were taken with a cork borer at the end of 2 and 4 hours. These samples were analyzed for the presence of viable Salmonella as previously described for the broiled and roasted chicken.

In an attempt to ascertain how great a health hazard exists at the present time in the cooked fowl being served in public eating establishments a total of 280 samples (229 chicken, 40 turkey, and 11 duck) were collected from dormitories, hospitals, and hotels and were examined for the presence of Salmonella. These samples included

fresh and frozen fowl which had been steamed, fried, fricasseed, or roasted by institutional methods.

From each whole chicken one sample was taken from the breast, one from the heavy inside musculature of the leg, and one from the tail region. From those chickens which were sectioned, only two samples were obtained: one from the breast and one from the heavy inside musculature of the leg. Although the samples were taken and treated in a manner almost identical with that described above for the cooked chicken, a somewhat different selection of plating media were used. Qualitative tests for the presence of viable Salmonella were made by preparing triplicate streak plates of the original ground sample on SS agar (Difco) and on MacConkey's agar. Quantitative tests were made by preparing triplicate pour plates of dilutions of the ground samples on tryptone glucose extract agar as well as the SS and MacConkey's agar. During the latter part of the study the use of the quantitative plates from selective media was discontinued since no Salmonella-like organisms seemed to appear on them, and only the use of the tryptone glucose extract agar was continued.

Representative colonies appearing on any medium were transferred to tryptone glucose extract agar slants or Krumweide triple sugar agar slants and studied further for fermentation reactions and response to the Gram stain.

RESULTS AND DISCUSSION

Table 1 summarizes the results obtained when chickens proven to have viable *Salmonella typhimurium* circulating in the blood stream were killed and cooked by broiling. The average weight of the chickens was 884 grams. Figure 1 shows the rate of heat penetration and the temperatures reached during such cookery. It is obvious that under conditions of these experiments broiling did not destroy 100 per cent of the viable Salmonella cells even though temperatures of 93-96°C. were attained.

TABLE 1

The Number of Viable Salmonella in Chicken Tissue Before and After Broiling [1]

Tissue	Before broiling		After broiling		
	Salmonella per gm.	Number of samples	Salmonella per gm.	Number of samples	Per cent reduction
Breast	3.75×10^9 ($1.0 \times 10^8 - 2.9 \times 10^{10}$)	13	9.05×10^7 ($0 - 1.37 \times 10^8$)	10	97.59
Leg	2.7×10^9 ($1.0 \times 10^8 - 2.0 \times 10^{10}$)	12	3.8×10^7 ($0 - 1.8 \times 10^8$)	8	98.59
Tail region	3.15×10^9 ($1.0 \times 10^8 - 2.48 \times 10^{10}$)	11	5.6×10^6 ($0 - 4.21 \times 10^8$)	10	99.82
Liver	1.1×10^9 ($9.0 \times 10^7 - 6.23 \times 10^9$)	15	1.24×10^6 ($0 - 5.26 \times 10^8$)	13	88.73

[1] Samples plated on SS agar after 24 hours incubation at 37°C. in tetrathionate broth.

Further amplification of the results presented in Figure 1 is possible if they are considered concurrently with the qualitative tests for the detection of viable Salmonella subsequent to having broiled the chickens. The qualitative tests were made by streaking portions of the undiluted suspension of chicken in tetrathionate broth, on SS agar. One hundred per cent of the plates so made from raw chicken yielded growth of Salmonella. The percentage of plates similarly made from the broiled chicken

which showed Salmonella colonies also was quite high, i.e., 81.2 per cent of those from the breast, 87.5 per cent of those from the leg, 81.2 per cent of those from the tail region, and 62.5 per cent of those from the liver. In no case was a Salmonella-infected chicken found to be completely free of viable cells subsequent to broiling. Salmonella were detected by the qualitative test plates at one or more sampling sites following the completion of cookery in every chicken.

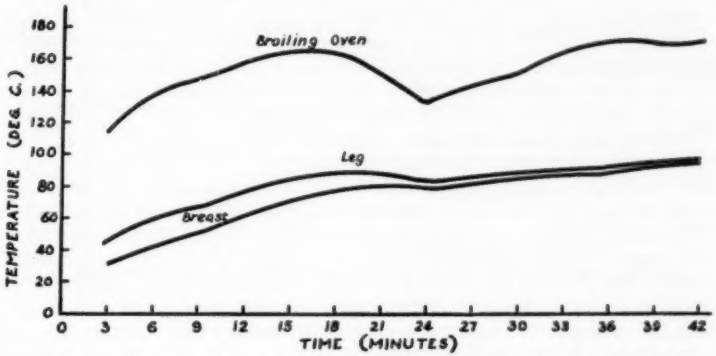

FIG. 1. Rate of heat penetration at the breast and at the leg of chicken during broiling (average of 16 determinations).

Thus, while the percentage of viable Salmonella cells destroyed by broiling was very high, in no case was an infected chicken rendered free of such bacteria under the conditions of these experiments.

Table 2 and Figure 2 present similar data for the roasting of chickens. The average weight of the roasting chickens was 1871 grams. In no case did roasting appear to destroy all of the viable Salmonella cells present in the chicken. This again was verified by the data obtained from qualitative tests. For all roasted chickens, growth of Salmonella was obtained from one or more of the areas sampled by use of SS agar (Difco) streak plates.

It should be pointed out that when Salmonella were detected on the qualitative test plates and appeared to be absent from all quantitative plates made from dilutions of the identical suspension of chicken or when colonies per plate were too numerous to count, such data were cast out in drawing up the averages for numbers of colonies per gram of chicken. When both qualitative and quantitative plates from a given specimen were negative, such results were included in making the numerical averages. Such a procedure seemed justified in that the positive qualitative tests indicated the organisms to be present and their absence from the quantitative plates indicated an improper degree of dilution rather than truly negative results. In further considering the numerical average figures for the quantitative study, it will be observed that the counts were made in all cases after a period of enrichment of the test samples in tetrathionate broth. Thus, the values for per cent reduction of cell count are

relative rather than absolute. The use of a period of tetrathionate enrichment for the test samples finds precedence in the work of Gibbons and Moore (1944) and Solowey and Calesnick (1948).

TABLE 2

The Number of Viable Salmonella in Chicken Tissue Before and After Roasting[1]

Tissue	Before roasting		After roasting		
	Salmonella per gm.	Number of samples	Salmonella per gm.	Number of samples	Per cent reduction
Breast	9.36×10^5 ($1.0 \times 10^6 - 9.551 \times 10^5$)	7	1.8×10^1 ($0 - 2.93 \times 10^4$)	7	99.9
Leg	7.31×10^5 ($3.3 \times 10^7 - 3.563 \times 10^5$)	7	1.96×10^4 ($0 - 7.03 \times 10^4$)	7	99.9
Tail region	3.99×10^5 ($1.9 \times 10^7 - 1.504 \times 10^5$)	7	2.02×10^4 ($0 - 4.35 \times 10^4$)	7	99.9
Liver	1.56×10^7 ($8.5 \times 10^6 - 9.307 \times 10^7$)	7	9.23×10^2 ($0 - 2.21 \times 10^4$)	7	99.9

[1] Samples plated on SS agar after 24 hours incubation at 37°C. in tetrathionate broth.

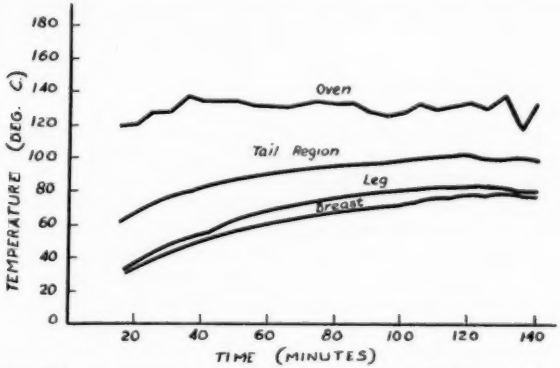

FIG. 2. Rate of heat penetration of the breast, leg, and tail region of chicken during roasting (average of eight determinations).

The implications regarding the effect of temperature on the destruction of Salmonella present in chicken muscle and liver seem to be fairly clear-cut if the data for the broiled chicken muscle only are considered. Apparently, the higher the degree of heat attained, the greater the percentage destruction of the viable Salmonella cells. The relationship is not so sharply defined when the roasting chickens are considered. The most probable explanation in these latter cases is the length of time of heating. Certainly neither broiling nor roasting chickens by presently recommended practices freed them completely of viable Salmonella cells when present. This was true in spite of the fact that in some cases the temperature was

above 100°C. for a period of time. The average maximum temperature for the broiled livers was 101.5°C. and for the roasted livers 93°C.

In experiments aimed at studying the effect of cookery on viable Salmonella in eggs, Gibbons and Moore (1944) reported the destruction of all Salmonella present by temperatures attained in scrambling eggs (3 minutes cooking time; maximum temperature of 82°C. inoculum in 2,000,000 to 5.600.000 cells per gram of dried egg powder), in cooking omelets (4 to 5 minutes, maximum temperature 94 to 98°C.), custards (internal temperature of custard not given), and in baking sponge cakes and muffins. Verder and Sutton (1933) fed custards heated to 62 to 72°C. to two persons and washings from these custards which had been heated to 62 to 64°C. for 30 minutes. None became ill although in one case the washings contained a few live organisms. Cathcart, Merz, and Ryberg (1942) reported that bringing starch-thickened "custards" to a boil rendered them free of Salmonella. Likewise, they found inoculated custard pie to be rendered sterile in relation to Salmonella by baking 25 to 35 minutes at 246°C. Not agreeing entirely with the above observations was the work of Solowey and Calesnick (1948) who found that scrambling Salmonella-contaminated eggs (temperatures to 75°C. were examined) could not be depended upon to free the eggs of the organisms. In general, the results presented in this paper indicate a persistence of the Salmonella cells at somewhat higher temperatures than has been previously shown. It must be noted, however, that the number of Salmonella cells used in the inoculation of the chicken was high.

In all cases the strains of Salmonella which were recovered from the cooked chickens were shown by the Typing Laboratory to be identical with the strains used for inoculations.

The somewhat limited study on the possibility of cross contamination to other foods from handling Salmonella infected chicken resulted in positive results in a large majority of the cases. Of ten samples on which plate counts were made at the end of 2 hours, the average number of Salmonella colonies per gram of liver sausage was 1.11×10^9 and of eleven samples held 4 hours was 5.89×10^8.

Turning to the survey made of cooked fowl served in public eating places, it was found that in no case was Salmonella recoverable from any sample. In general, about 50 per cent of the samples of chicken, duck, or turkey yielded growth on tryptone glucose extract agar but neither on this medium nor on the more selective media were Salmonella organisms detected. The percentage of samples yielding growth on the selective media was quite low (0-16.6 per cent on SS agar; 0-21.4 per cent on MacConkey agar). The organisms recovered from the selective media appeared to be coliforms but the study of them was not extended.

There is no basis for knowing whether the chickens, ducks, and turkeys examined were Salmonella-free or whether the cookery employed rendered them sterile of these organisms. The methods used in preparation of the fowl analyzed in this survey include frieasseeing, frying, steaming, and roasting. In all cases the report of the chef indicated the application of high temperature over relatively long periods of time. The ducks and

turkeys were roasted at much higher temperatures than those currently suggested as giving the most desirable quality of cooked meat. For example, the ducks and turkeys were roasted throughout their period of cookery at 450°F.(232°C.). This would mean that such poultry would be lacking in juice and flavor. It is possible that longer cooking at somewhat lower temperatures might result in fowl of both increased palatability and equal safety.

SUMMARY

The possibility of food poisoning resulting from the transmission of Salmonella from infected chicken to man was examined. A consideration of broiling and roasting as cookery methods which might destroy the Salmonella and render the chicken safe was emphasized. The problem was further studied by analyzing samples of cooked fowl secured from public eating establishments for Salmonella.

The results indicate that currently accepted methods of broiling or roasting markedly reduce the number of Salmonella present in chicken muscle or liver. However, in no case was a chicken rendered sterile of Salmonella, by cookery methods used in these experiments.

Of 280 samples of cooked fowl collected from public eating establishments, none were found to contain viable Salmonella. High temperatures during cookery were reported to have been used in all cases. This procedure or the fact that the chickens were Salmonella free previous to cooking may explain the negative results.

Contamination of other foods resulting from previous handling of diseased chicken appears to be a possibility.

REFERENCES

BEISTER, W. E., AND DEVRIES, R., 1943. Diseases of Poultry. The Iowa State College Press, Ames, Iowa. pp. 177.

BORMAN, E. K., WHEELER, K. M., WEST, D. E., AND FICKLE, F. L., 1943. Salmonella typing in a public health laboratory. Am. J. Pub. Health, **33**, 127-134.

BORNSTEIN, S., 1943. The state of the Salmonella problem. J. Immunol., **46**, 439-496.

BUNYEA, H., 1946. Use of the rapid whole-blood test for pullorum disease. U. S. Dept. Agric. Publ. **349**.

CATHCART, W. H., MERZ, A., AND RYBERG, R. E., 1942. Staphylococci and Salmonella control in foods. IV. Effect of cooking bakery custards. Food Research **7**, 100-103.

CHERRY, W. B., SCHERAGO, M., AND WEAVER, R. H., 1943. The occurrence of Salmonella in retail meat products. Am. J. Hyg., **37**, 211-215.

EDWARDS, P. R., 1939. Incidence of *Salmonella* types in fowls in the United States. Proc. Seventh World's Poultry Congress, 271-274.

———, AND BRUNER, D. W., 1943. The occurrence and distribution of Salmonella types in the United States. J. Infectious Diseases **72**, 58-67.

———, ———, AND MOROW, A. B., 1948. Salmonella infections of fowls. Cornell Vet., **38**, 247-256.

GIBBONS, N. E., AND MOORE, R. L., 1944. Dried whole egg powder. XII. The effect of drying, storage and cookery on the Salmonella content. Can. J. Research **22F**, 58-63.

HINSHAW, W. R., MCNEIL, E., AND TAYLOR, T. S., 1944. Avian Salmonellosis. Types of Salmonella isolated and their relation to public health. Am. J. Hyg., **40**, 264-278.

JUNGHERR, E., 1940. Paratyphoid infections in birds. Vet. Med., **35**, 112-116.

MYERS, W. A., 1946. Gastroenteritis aboard ship. Public Health Reports, **61**, 1853-1858.

MOSHER, W. E., WHEELER, S. M., AND HARDY, A. V., 1941. Studies of acute diarrheal diseases. V. An acute outbreak due to *Salmonella typhimurium*. Public Health Reports, **55**, 2415-2426.

RETTGER, L. F., HULL, T. G., AND STURGES, W. S., 1916. Feeding experiments with Bacterium pullorum. The toxicity of infected eggs. J. Exp. Med., **23**, 475-489.

SOLOWEY, M., AND CALESNICK, E. J., 1948. Survival of *Salmonella* in reconstituted egg powder subjected to holding and scrambling. Food Research, **13**, 216-226.

VERDER, E., AND SUTTON, C. J., 1933. Is Salmonella food poisoning caused by living bacilli or by thermolabile toxic products? J. Infectious Diseases, **53**, 262-271.

THE GROWTH INITIATION OF BACTERIA IN DEFROSTED EGGS[1,2]

S. E. HARTSELL
Purdue University, Lafayette, Indiana

[Received for publication, May 29, 1950]

The problem of how bacteria increase after defrosting a frozen food seems not to have been investigated extensively. A voluminous literature is available relative to the total number of viable cells in frozen foods immediately after defrosting, but little attention has been given to later intervals of sampling and to the food requirements of the surviving cells. Standard procedures are usually employed by the control laboratory, hence there is little concern for such experimental studies. Those persons responsible for the microbiological aspects of the quality control of a food adopt plant practices to insure satisfactory results. This is as it should be. However, the constant study and improvement of laboratory procedures may permit even more representative results than were formerly obtained.

In this study, the growth characteristics of bacteria that survived storage in frozen eggs were investigated. The work was predicated upon the belief that, if pathogenic forms were present in a food, their potential health hazard should be known, even though modern plant practices and the bactericidal effect of the freezing process minimize the danger of infecting the consumer. The longevity and behavior of *Micrococcus pyogenes* var. *aureus*, *Shigella dysenteriae*, and a non-pathogenic organism, *Escherichia coli*, was studied at various subfreezing temperatures.

Since Dyar and Keith (1893) first suggested *E. coli* as an indicator of polution, it has often been the test species when studying the the effect of temperature on bacteria. That *E. coli* and the coliform group could survive in frozen eggs has been shown by many investigators: Brownlee and James (1939), Colien (1942), Holtman (1943), Johns and Berard (1946), Nielson and Garnatz (1940), Pennington (1941), Quinn and Garnatz (1943), Schneiter, Bartram, and Lepper (1943), Sherman and Naylor (1942), and Wallace and Park (1933). In previous studies it has been observed that a rapid reduction in viable cells occurred in the early periods of exposure and storage at subfreezing temperatures; however, viable cells were present in some samples after 18 months.

E. coli was also the test species used in describing the effects of cold temperatures on the culture when still within the growth range, by Hegarty and Weeks (1940), Sherman and Cameron (1934), and Sherman and

[1] This investigation was supported (in part) by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service. The author gratefully acknowledges, also, the assistance of the Purdue Research Foundation while doing this research.

[2] Presented at the Tenth Annual Meeting of the I.F.T., Chicago, Illinois, May 23, 1950.

Naylor (1942). In these studies a "physiological rejuvenescence" of surviving cells was described as a part of the explanation of lag in the culture. The ability of *E. coli* to survive extremes of subfreezing temperatures permitted other investigators, Haines (1935) and (1937-38), Hampil (1932), Hershey (1939), Hilliard and Davis (1918), Lipman (1937), Monod (1949), Weiser and Hargiss (1946), Weiser and Osterud (1945), and Yudkin (1937), to elucidate some of the principal effects, both physical and chemical, that subfreezing temperatures have on bacteria. However, in these investigations the medium was one of the constant factors and apparently was not varied experimentally, though tests for viability were the criteria upon which the conclusions were based.

Curran and Evans (1937) and Nelson (1943) have drawn attention to the concept that bacteria or their spores when subjected to extremes of physical or chemical environment are more demanding in their nutritional requirements. Gunderson and Rose (1938) found that violet-red bile agar is very inhibitory to coliform bacteria in frozen foods; hence microbial standards for judging the quality of the product must take this into consideration. Presumably, investigations on the longevity and behavior of this group at subfreezing temperatures should utilize other media which are more optimal for these organisms than some of the selective media currently available. Goresline (1946) has indicated the superiority of glucose tryptone agar over other plating media when determining the viable bacterial count of frozen vegetables. Hartsell (1944) has shown that yeast water added to glucose tryptone agar is a desirable medium for determining the total bacterial counts of stored, spray-dried, whole-egg powder. In the present investigation further evidence is presented for the use of such a highly nutritive substance, when detecting the viability of cells having been exposed to severe physical conditions.

The hardiness of *M. aureus*, another test species in this study, at subfreezing temperatures has been observed in the investigations of Gunderson and Rose (1948), Haines (1935), Jones and Lochhead (1939), Lochhead and Jones (1938), and McCleskey and Christopher (1941). The latter investigators and Wallace and Park (1933) reported that *S. aertrycke*, the third test species, could survive when held at —18°C. for several months.

In the present paper observations are reported on the ability of these bacteria to initiate growth after having been frozen. In addition, results are presented on their survival at various temperatures, on the influence certain media have on surviving cells, and on the correlation of these observations with the time of sampling.

METHODS

The preparation of the egg-culture mixture, prior to freezing, was as follows: Fresh eggs, never more than 24 hours old, were candled, immersed in a 0.2 per cent solution of bichloride for 15 minutes, then into 7 per cent tincture of iodine momentarily, air dried, and broken into a Waring Blendor, mixed for three seconds and allowed to stand for ten minutes. A suspension of the test species was made in phosphate buffer, pH 7.0, from the growth on an 18- to 24-hour yeast extract-veal infusion agar slant. The suspension was then standardized to a reading of 70 on the Cenco-Sheard Photelometer. Different amounts of the suspension, according to the demands of the experiment, were then introduced into the liquid eggs. The mixture was blended

for five seconds and ten ml. were transferred to sterile test tubes. Platings were made immediately to obtain the initial counts. Since these studies were experimental with regards to the media to be used in detecting viable cells, a "control" medium of yeast extract-veal infusion agar was used. This medium, hereafter designated YE-VI agar, was composed of 100 ml. of yeast water, 100 ml. of veal infusion, 0.5 per cent tryptone, 0.5 per cent proteose peptone, 0.5 per cent sodium chloride, 2 per cent agar, and water. The medium was adjusted to pH 7.0. The experimental plating media were MacConkey agar for *E. coli* and *S. dysenteriae*, and Staphylococcus Medium No. 110 (Difco) for *M. aureus*.

The eggs were quick frozen at −25°C. for four hours, then transferred to storage temperatures of −1°C., −9°C., or −17.8°C., according to the demands of the experiment. Defrosting and holding of the tubes for growth initiation studies was in a 37°C. water bath. After five minutes the eggs were completely defrosted and plates of appropriate decimal dilutions were poured in triplicate. Total counts were made after 48 hours of incubation at 37°C.

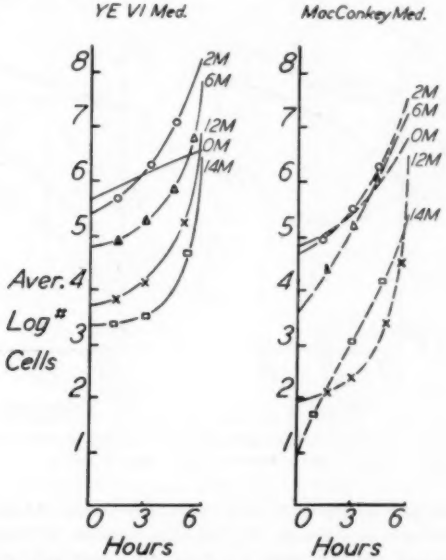

FIG. 1. Growth initiation of *E. coli* after storage at −9°C. "M" signifies months of storage, "Hours" the period of time after thawing.

RESULTS

The total plate counts for *E. coli*, as represented in Figure 1, show that YE-VI agar was much more satisfactory than MacConkey agar when detecting viable cells after storage of this culture at −9°C. for many months. The toxic effect of yeast extract, noted by Curran and Evans (1937) when testing the viability of heat-treated spores, was not evident in these experiments. Perhaps it had been neutralized by some component of the veal infusion or of the defrosted eggs.

On the other hand, MacConkey agar was inhibitory to the surviving cells when the sample was plated immediately after defrosting; yet this

medium possessed some constituents which favored the growth of cells three and six hours later. The abrupt rise in total cell count, particularly six hours after defrosting the cultures stored for six, 12, and 14 months, indicates an especially suitable medium for the surviving cells.

After two and six months of storage, both media showed higher counts for the culture six hours after defrosting and holding at 37°C. than were obtained with the initial (0-month) plating. Apparently the subfreezing temperature altered the physiological status of the surviving cells so that a much shorter generation time occurred. The reason for this stimulus is not too evident. In any case, the same types of curves were obtained at

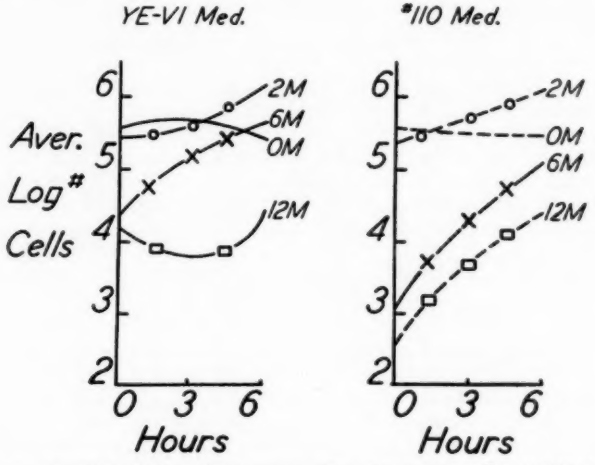

FIG. 2. Growth initiation of *M. aureus* after storage at −9°C. "M" signifies months of storage, "Hours" the period of time after thawing.

the intervening plating periods with two other levels of initial population held under identical conditions for the same periods of time. Apparently the "physiological rejuvenation" of bacteria, described by Sherman and his colleagues (1934, 1942) for chilled cultures of *E. coli*, can occur in cultures which have been frozen and stored at subfreezing temperatures. The stimulative effects of the environmental conditions used in this study have been noted in several species of bacteria, with varying levels of initial population and at different temperatures of storage. *Salmonella oranienburg*, *Salmonella typhosa*, and *Salmonella aertrycke* were affected in the same manner as *E. coli*.

M. aureus, when plated on the control medium and the selective medium No. 110, again indicated the superiority of YE-VI agar when testing the viability of frozen pure cultures of bacteria in whole, liquid eggs (Figure 2). However, the bacteriostatic action of salt in this medium would make it very practical when testing commercial samples of frozen

eggs because of its inhibitory effect on many associated bacteria. The stimulus of the cold temperatures to the generation time of the defrosted cells is again evident, as well as the very hardy nature of this species when stored at −9°C.

S. dysenteriae was much more sensitive to the conditions of these experiments. Apparently growth in the unfrozen liquid eggs was difficult for this organism (Figure 3). Tests for the susceptibility of this strain

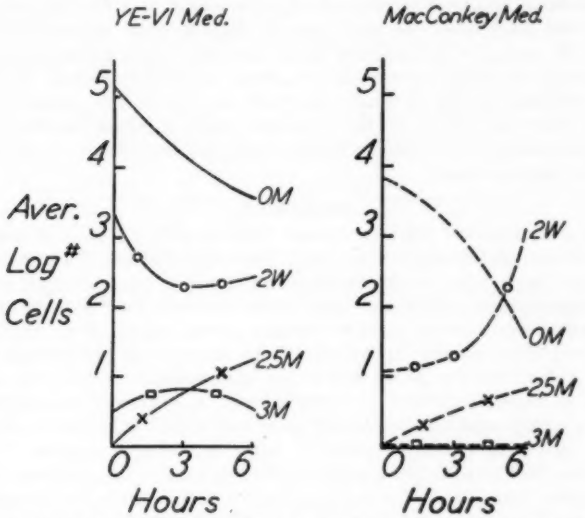

FIG. 3. Growth initiation of *S. dysenteriae* after storage at −9°C. "M" signifies months of storage, "Hours" the period of time after thawing.

to the lysozyme of egg-white indicated that it was insensitive. Other factors must have accounted for the early evidence of low viability. The results indicate that, after 2.5 months of storage, adaptation of the culture to some extent had occurred. Six hours after defrosting a slight stimulus to cellular growth was evident. The abrupt rise in the growth curve, as discerned from the counts on both media, indicates that the same type of stimulus noted with *E. coli* occurred in these cultures. The inhibitory influence of MacConkey agar, and the superiority of YE-VI agar, at the three-month storage interval clearly indicates that platings made immediately after defrosting were not representative of the viable cells present in these tubes. Yet this medium afforded relatively optimum conditions for growth of *S. dysenteriae* three and six hours after defrosting.

Since the analysis of the data on the longevity of the cultures was made before the analysis of the behavior after defrosting, it was of interest to inquire how soon after freezing and storage this phenomenon could

be observed. Figure 4 shows that *E. coli* evidenced this change after one day of storage at —9°C. The stimulation reached a maximum after one month of storage and was still evident after two months, particularly six hours after defrosting. This was true in both media, even though a lower level of viable cell population was detected in MacConkey agar.

It is common knowledge that "sharp" freezing injures the bacterial cell very little. Storage of the test species at —18°C. showed very little change in the early growth curve of the experimental cultures as well as the control. *E. coli* was stimulated after storage at this temperature (Figure 5) and manifested the same type of growth curve as when stored at —9°C. *M. aureus* remained very much the same in its growth pattern at all intervals of sampling throughout seven months of storage. *S. dysenteriae* initiated growth as usual. However, the zero count, obtained immediately after defrosting of the 12-month culture, again emphasizes the misrepresentative evidence of viability that can be obtained with certain selective plating media.

DISCUSSION

The data indicate that the culture medium and the time of sampling after defrosting frozen eggs are very important factors if representative total plate counts are to be obtained. A medium such as yeast extract-veal infusion agar, which has considerable quantities of essential metabolites, will permit frozen cells to initiate growth much more easily than selective plating media. It has also been shown that the pathogens, *M. aureus* and *S. dysenteriae*, as well as the non-pathogen, *E. coli*, will survive storage in frozen eggs held for many months at subfreezing temperatures.

MacConkey agar was inhibitory to *E. coli* and *S. dysenteriae* when the stored eggs were plated immediately after defrosting; however, this medium provided satisfactory growth conditions for the culture three and six hours after defrosting. The explanation for this result is not immediately apparent. Since the bacteria were stored in frozen eggs, where they had become accustomed to a low surface tension, the bile salts in MacConkey agar may have provided optimal conditions of osmotic pressure for the generating cells, particularly a few hours after being defrosted. This factor was observed by Levitt and Scarth (1936) to be very important in the frost-hardening of living plant cells.

The presence of a readily fermentable carbohydrate, lactose, might account for a part of the rapid generation rate noted when *E. coli* grew on MacConkey agar. However, the YE-VI medium, which did not contain lactose, reflected the same rapid rise in cell count in the early growth phases. Presumably the vitamin demands of the frozen cells were rather easily satisfied in this medium, because a consistently higher level of total plate count was obtained at all sampling intervals.

Staphylococcus medium No. 110 was not as effective in growing *M. aureus* after the latter had been stored for six and 12 months in frozen eggs; however, it was not inhibitory to the generations of cells three and six hours after defrosting. Apparently this bacterium requires a physical or nutritive environment, as represented in YE-VI agar, which is much more optimal in vitamins and trace elements.

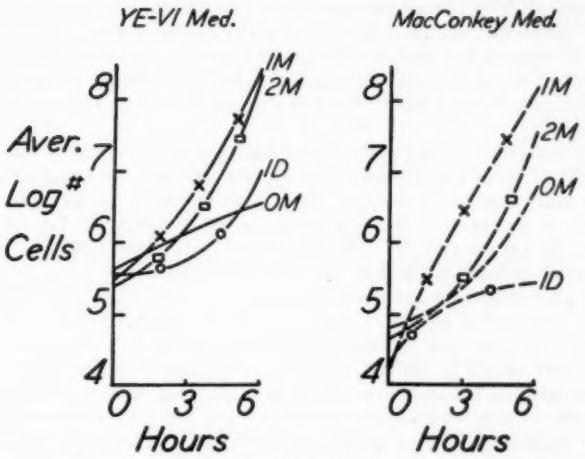

FIG. 4. Early evidence of the stimulation of *E. coli* by a temperature of −9°C. "D" signifies days, "M" months of storage, "Hours" the period after thawing.

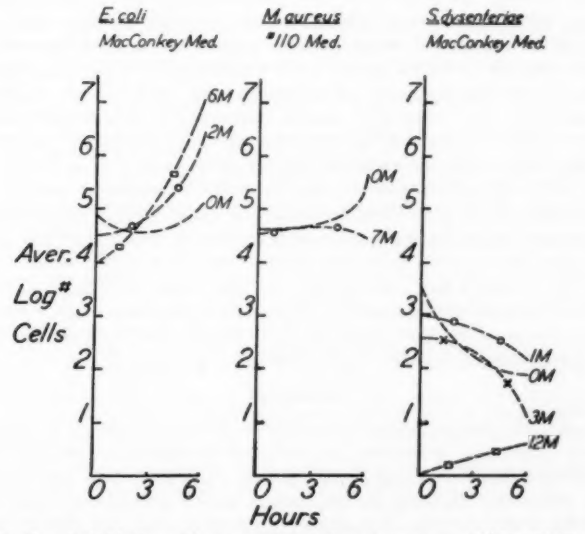

FIG. 5. Growth initiation of test organisms after storage at −18°C. "M" signifies months of storage, "Hours" the period after thawing.

E. coli, after thawing, though reduced in numbers of viable cells as storage continued, initiated growth more vigorously than the unfrozen control. *S. dysenteriae* and *M. aureus* did likewise. This was evident from the counts of the control medium and the selective medium, which reflected a stimulus to greater metabolic activity to the extent of a shortened generation time.

S. dysenteriae was not particularly vigorous in its viability under the conditions of these experiments, but showed the same stimulus after two and one-half months of storage. Thus, the activation to the generation processes of bacteria at freezing temperatures appears in the relatively resistant *M. aureus* and the relatively sensitive *S. dysenteriae*, as well as the moderately vigorous *E. coli* and the *Salmonella*.

The physiological stimulation, observed in these studies as occurring in frozen eggs stored at −9°C., also occurred in populations at −1°C. and −17.8°C. The contrast in the growth initiation of the different cultures was greatest at −9°C. where, curiously, the greatest reduction in viable cells was noted. The vigorous physical alterations of the colloidal state of the cells at this temperature apparently are beneficial to surviving bacteria if the changes are reversible, and suitable nutrients are available in the form of plating media.

Hershey (1939) questioned the observations of Curran and Evans (1937) that enrichment substances were necessary for accurate enumeration of bacteria which have been exposed to highly lethal physical or chemical factors. He considered that provision of a liquid state about the surviving cells would create physical conditions much more conducive to growth of the culture. A more accurate enumeration of viable cells would thus be obtained if liquid media were available for the determination of the Most Probable Numbers of a culture. In view of the experiments reported above, the suggestion seems particularly appropriate that considerable effort be directed toward the provision of better methods for detecting viable coliform bacteria and potential pathogens in frozen foods.

The above data also indicate that an extended series of studies on the practical aspects of defrosting frozen foods might show that, if pathogens were present, more representative results would be obtained, at least in frozen eggs three to six hour after defrosting. This would be particularly true if the product had been stored for very long periods of time. Plating of such samples immediately after defrosting gave entirely erroneous results; viable cells were present, but needed time and an appropriate plating medium to indicate their presence.

SUMMARY

E. coli, *M. aureus*, and *S. dysenteriae* were observed to be stimulated in their generative processes in liquid whole eggs by "sharp" freezing and storage at subfreezing temperatures. The increased rate of binary fission was observed early in the storage period and occurred in cultures stored for many months. The need for a highly nutritive plating medium, such as yeast extract-veal infusion agar, for bacterial cells exposed to such physical conditions has been shown. If more representative plate counts are desired the use of such a medium is clearly indicated. The selective

medium, MacConkey agar, was inhibitory to *E. coli* and *S. dysenteriae* when first defrosted, but was satisfactory after the culture had been incubated for a few hours. This medium reflected a stimulus to rapid cellular generation in the later storage periods, presumably by reason of some physical and chemical factors peculiar to the influences of subfreezing temperatures. The importance of the sampling time of defrosted eggs suggests the need for further studies, if representative results are desired when using selective plating media for the cultivation of *E. coli*, *M. aureus*, and *S. dysenteriae* from frozen eggs.

Acknowledgments

The author is very grateful for the technical assistance of Mrs. Ellen Robbins throughout this research.

REFERENCES

BROWNLEE, D. S., AND JAMES, L. H., 1939. Bacterial contamination of frozen whole eggs and an improved method of defrosting. World Poultry Cong. Proc. 488-492.

COLIEN, F. E., 1942. The microbiology of frozen eggs. I. Presence and significance of coliform organisms in frozen eggs. J. Bact., **42**, 47.

CURRAN, H. R., AND EVANS, F. R., 1937. The importance of enrichments in the cultivation of bacterial spores previously exposed to lethal agencies. J. Bact., **34**, 178-189.

DYAR, H. G., AND KEITH, S. C., 1893. Notes on normal intestinal bacilli of the horse and of certain other domesticated animals. Technology Quart., **6**, 256-257.

GORESLINE, H. E., 1946. Microbiological examination of foods: Tentative methods for microbiological examination of frozen foods. Report of the committee on standard methods for the microbiological examination of foods. Am. J. Pub. Health, **36**, 332-335.

GUNDERSON, M. F., AND ROSE, K. D., 1948. Survival of bacteria in a precooked, fresh frozen food. Food Research, **13**, 254-263.

HAINES, R. B., 1935. The freezing and death of bacteria. Ann. Rept. Food Invest. Board, 31-34.

———, 1937-38. The effect of freezing on bacteria. Proc. Roy. Soc. (London), **124B**, 451-463.

HAMPIL, B., 1932. The influence of temperature on the life processes and death of bacteria. Quart. Rev. Biol., **7**, 172-196.

HARTSELL, S. E., 1944. Studies on the bacteriology of stored, dried egg powder. Food Research, **9**, 505-511.

HEGARTY, C. P., AND WEEKS, O. B., 1940. Sensitivity of *Escherichia coli* to cold-shock during the logarithmic growth phase. J. Bact., **39**, 475-484.

HERSHEY, A. D., 1939. Factors limiting bacterial growth. VII. Respiration and growth properties of *Escherichia coli* surviving sublethal temperatures. J. Bact., **38**, 563-578.

HILLIARD, C. M., AND DAVIS, M. A., 1918. The germicidal action of freezing temperatures upon bacteria. J. Bact., **3**, 423-431.

HOLTMAN, D. F., 1943. The bacteria of fresh and frozen eggs in relation to public health. J. Bact., **45**, 50.

JONES, A. H., AND LOCHHEAD, A. G., 1939. A study of micrococci surviving in frozen-pack vegetables and their enterotoxic properties. Food Research, **4**, 203-216.

JOHNS, C. K., AND BERARD, H. L., 1946. Effect of freezing and cold storage upon the bacterial content of egg melange. Sci. Agr., **26**, 34-42.

LEVITT, J., AND SCARTH, G. W., 1936. Frost-hardening studies with living cells. I. Osmotic and bound water changes in relation to frost resistance and the seasonal cycle. Can. J. Research, **14**, 267-284.

LIPMAN, C. B., 1937. Tolerance of liquid air temperatures by spore-free and very young cultures of fungi and bacteria growing on agar media. Bull. Torrey Bot. Club, **64**, 537-546.

LOCHHEAD, A. G., AND JONES, A. H., 1938. Types of bacteria surviving in frozen-pack vegetables. Food Research **3**, 299-306.

MCCLESKEY, C. S., AND CHRISTOPHER, W. N., 1941. Some factors influencing the survival of pathogenic bacteria in cold-pack strawberries. Food Research, **6**, 327-333.

MONOD, J., 1949. The growth of bacterial cultures. Ann. Rev. Microbiol., **3**, 371-394.

NIELSON, F. C., AND GARNATZ, G. F., 1940. Observations on the death rates for the bacterial flora of frozen egg products. Proc. Inst. Food Technologists, 289-294.

NELSON, F. E., 1943. Factors which influence the growth of heat-treated bacteria. I. A comparison of four agar media. J. Bact., **45**, 395-403.

PENNINGTON, M. E., 1941. The freezing of eggs. Refrig. Eng., **42**, Sect. 2, December.

QUINN, H., AND GARNATZ, G. G., 1942. A study of the increase of bacterial counts of frozen whole eggs, using three methods of thawing. J. Bact., **45**, 49-50.

SCHNEITER, R., BARTRAM, M. T., AND LEPPER, H. A., 1943. Bacteriological and physical changes occurring in frozen eggs. Influence of defrosting and prolonged storage on bacterial count and on odor. J. Assoc. Official Agr. Chem., **26**, 172-173.

SHERMAN, J. M., AND CAMERON, G. M., 1934. Lethal environmental factors within the natural range of growth. J. Bact., **27**, 341-348.

———, AND NAYLOR, H. B., 1942. Aging without reproduction and the viability of young bacterial cells at low temperatures. J. Bact., **42**, 749-756.

WALLACE, G. I., AND PARK, S. E., 1933. Microbiology of frozen foods. IV. Longevity of certain pathogenic bacteria in frozen cherries and in frozen cherry juice. J. Infectious Diseases, **52**, 146-149.

WEISER, R. S., AND HARGISS, C. O., 1946. Studies on the death of bacteria at low temperatures. II. The comparative effects of crystallization, vitro-melting, and devitrification on the mortality of *Escherichia coli*. J. Bact., **52**, 71-79.

———, AND OSTERUD, C. M., 1945. Studies on death of bacteria at low temperatures: Influence of intensity of freezing temperatures, and period of exposure to freezing temperatures on mortality of *Escherichia coli*. J. Bact., **50**, 413-439.

YUDKIN, J., 1937. Cell structure and enzymic activity. Biochem. J., **31**, 1065-1068.

SOME EFFECTS OF PROCESSING ON THE NUTRITIVE PROPERTIES OF PROTEINS[1]

R. R. BALDWIN, J. R. LOWRY, AND REINHARDT THIESSEN, JR.
Central Research Laboratories, General Foods Corp., Hoboken, N. J.

[Received for publication, May 29, 1950]

The processing of foods is a large and important industry and has become a necessity in our modern way of life. Through processing, the excess from one harvest may be carried over to the next and perishable foods may be served out of season. In addition, the processing of foods often makes them more acceptable to the consuming public. Flavors and palatability are often improved and in some cases the food value of the nutrient constituents is also increased.

On the other hand, some food processing methods are deleterious. Perhaps the most widely recognized food nutrients which are destroyed to various degrees by commercial processing as well as by home cooking are the vitamins. Less recognized but just as important is the loss in protein nutritive quality. Although the deleterious effects of heat treatment on proteins have been known for a long time, it is only lately that this important problem has received the attention that it merits.

Our interest has been centered around the effects of heat on protein nutritional quality and more specifically on the effects of heat on proteins when reducing sugars such as dextrose are present during the processing.

The reaction that takes place is apparently that of the nonenzymatic browning (Maillard) reaction between the free amino groups of the protein and the reducing sugar. This reaction is important in that the storage life of many foodstuffs is shortened because of this reaction which causes darkening, flavor changes, and sometimes formula failure. There is evidence now to indicate that the nutritive value of the protein is also impaired. Likewise, during the processing of some food materials such a reaction also occurs, resulting in color and flavor changes, and more important, in nutritional changes which have only recently been recognized.

METHODS

Preparation of Protein-dextrose Complexes. In these experiments casein[2] was used as the protein. Unless otherwise stated the casein-dextrose complexes were prepared by mixing one gram of casein with one gram of dextrose[3] per gram of pure protein (N × 6.38) and 0.5 ml. of water per gram of casein, and autoclaving for the desired period and at the designated temperature. Usually one-kilo batches were prepared in shallow Pyrex pans.

Animal Assay Methods. Diets were prepared from treated and untreated casein so that the protein content was approximately ten per cent and fat was added to six per cent. There was a full complement of vitamins and minerals, and starch was added

[1] A part of this paper was presented before the Tenth Annual Meeting of the I.F.T., Chicago, Illinois, May 22, 1950.

[2] General Biochemicals, Inc. [3] Merck's anhydrous.

to make the diets isocaloric. Cod liver oil was administered twice a week by mouth. The composition of the diets is shown in Table 1.

TABLE 1

Composition of the Diets Used

Mazola corn oil	60.0	gm.
Salts [1]	40.0	gm.
Choline chloride	1.0	gm.
α-Tocopherol acetate	0.1	gm.
Pyridoxine HCl	0.02	gm.
Calcium pantothenate	0.05	gm.
Riboflavin	0.05	gm.
Thiamine HCl	0.012	gm.
Nicotinic acid	0.09	gm.
Biotin	0.0003	gm.
Folic acid	0.0009	gm.
Inositol	0.20	gm.
PABA	0.30	gm.
2-Methyl-1,4-napthoquinone acetate	0.002	gm.
Protein, to make 10 per cent		
Corn starch, to make up to	1,000	gm.

[1] U.S.P. XII (Nutritional Biochemicals).

The animals were housed in individual raised-bottom wire cages in an air-conditioned room at 24°C. Water was offered *ad libidum*. Male, weanling, albino rats, 28-29-days old were usually used, six to a group. For some tests five males and five females were used. Animals were distributed so that the average weights of the respective groups were comparable. The paired feeding technique was used, the group of animals eating the least being used to control the food intake of the other groups. The animals were weighed three times a week. The duration of the tests was 28 days.

Carbohydrate Binding. Ten grams of the material were placed in a glass tube, 12 mm. inside diameter and 14 inches long. The bottom four inches of this tube were constricted to four mm., i.d. At this constriction a wad of glass wool was placed and the sample poured in on top. The constricted end was fitted through a stopper and placed in a three-liter filtering flask. Distilled water was allowed to percolate through the sample until negligible solids were leached out. From time to time the sample was mixed to prevent channeling.

At the end of the extraction the samples were dried and analyzed for nitrogen. The per cent bound carbohydrate was then calculated as follows:

(N of untreated casein — N of extracted complex/N of untreated casein) × 100.

Determination of Reactive Amino and Carboxyl Groups (Dye-binding). The determination of reactive free amino groups and carboxyl groups was carried out by a modification of the method of Fraenkel-Conrat and Cooper (1944).

The proteins were ground in a Raymond mill and sifted for three minutes on silks. The size fraction between 6xx and 8xx was used.

Ten milligrams of sifted protein (N × 6.38) were weighed into each culture tube (15 × 150 mm.) and two glass beads were added. Eight dye concentrations per sample were found to give a suitable spread and seven were needed for the blank. Each sample was run in duplicate. To each tube was added one mil. of buffer and varying amounts of dye (0.5 ml. to 17.0 ml. for the samples, and 0 to 20.0 ml. for the blank).

The tubes were stoppered and shaken at room temperature (25-30°C.) 24 hours. Tubes which were cloudy were centrifuged and one ml. of clear supernatant from each tube was diluted to 100 ml. The diluted solutions were used to double rinse and fill Klett tubes for measurement of light transmission in a Klett-Summerson photoelectric colorimeter equipped with blue filter No. 42.

The "Dye-binding Constant" was calculated by determining the X axis intercept by the method of least squares, using all values of Y (Klett reading) which were above the point of inflection.

Microbiological Assay of Essential Amino Acids. The usual procedures were used for the microbiological assay of the essential amino acids. For digestion, sufficient sample to make 50 mg. of nitrogen was weighed into a six-inch Pyrex test tube. Ten ml. of 6 N HCl was then added, the tubes sealed off, and the contents digested for 36 hours at 15 p.s.i. The digests were made up to 50 ml., and filtered. Aliquots were then diluted to the appropriate volumes. For tryptophan 5 N NaOH was used for hydrolysis.

The medium described by Horn, Jones, and Blum (1946) was used for all amino acids, and in general their methods were used throughout. For lysine, methionine, phenylalanine, and histidine, *L. mesenteroides* (P60) was used. For threonine, tryptophan, leucine, isoleucine, valine, and arginine, *S. faecalis* was used. In all cases both pyridoxine and pyridoxamine were added to the media.

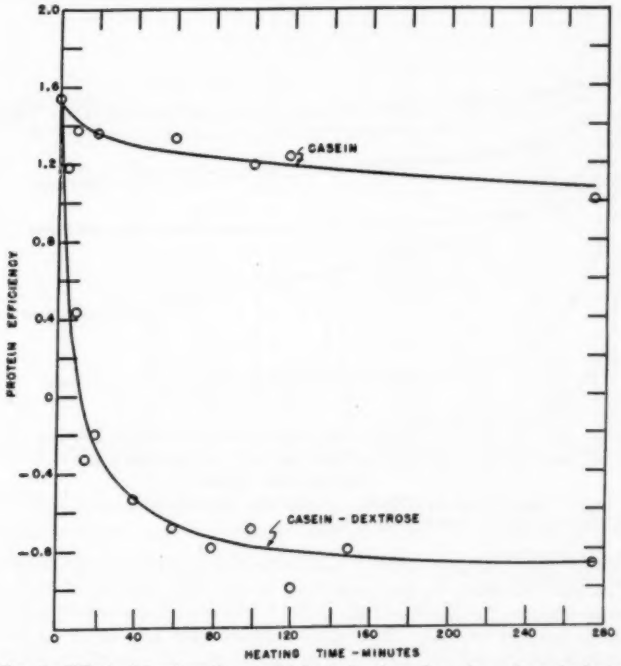

FIG. 1. Effect of heating time on biological value of casein and casein-dextrose heated at 15 p.s.i.

RESULTS AND DISCUSSION

McInroy, Murer, and Thiessen (1949) previously showed that heating casein in the presence of dextrose reduced the biological value of the protein to a far greater extent than did heating the casein in the absence of the reducing sugar. These studies have now been extended to include more conditions.

The effect of the duration of heating casein with dextrose upon the biological value of this protein is shown in Figure 1. The reaction at 15 p.s.i. (121°C., 250°F.) is extremely rapid. After only three minutes of heating

the nutritive value of the protein is already impaired to about 25 per cent. After 15 minutes the protein is so severely damaged that it will no longer maintain weight, resulting in a negative protein efficiency. As heating is continued up to about 80 minutes, the nutritive value of the casein continues to decline, with further weight losses in the animals. After this period, the protein efficiency levels off and there is only a very slight further loss up to the 275-minute period. Almost identical results were obtained when wheat gluten was autoclaved with dextrose.

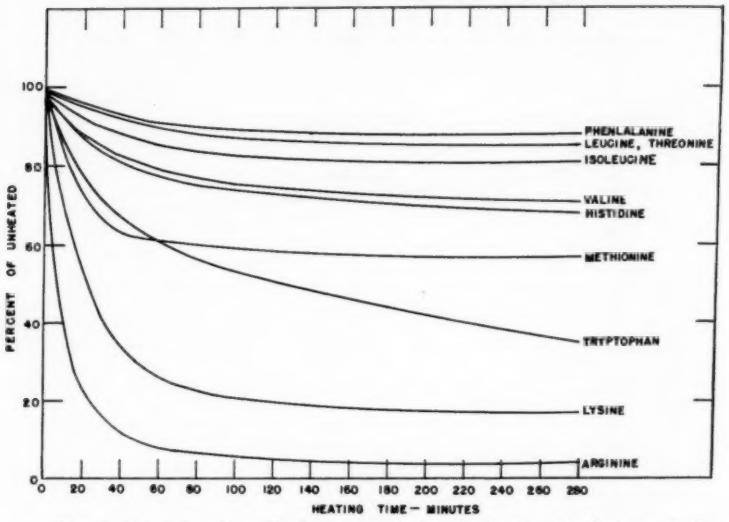

FIG. 2. Loss of amino acids in progressively autoclaved casein-dextrose at 15 p.s.i. Determined by microbiological assay.

Lowry and Thiessen (1950) had indicated that this impairment was due, in part at least, to the reduction in the digestibility of the casein-dextrose complex by enzymes "in vitro." The complexes were particularly resistant towards trypsin. New data indicate that a greater part of the nutritive loss is more probably due to destruction of essential amino acids. By destruction of amino acids we imply that the essential amino acids of the protein are physiologically unavailable to the test organism.

The heating of casein with dextrose results in a high degree of destruction of the essential amino acids. Figure 2 shows the rapidity with which some of the essential amino acids are destroyed as determined by microbiological assays. Arginine and lysine are particularly susceptible, with tryptophan and methionine next in order, followed by histidine and valine. Phenylalanine, leucine, isoleucine, and threonine are not affected to any appreciable extent. Both lysine and arginine are diamino acids and with the α-amino group tied up in the peptide linkage both will have another amino group free to react with the carbohydrate.

Comparison of the curves for lysine and arginine in Figure 2 with that of the biological value curve in Figure 1 demonstrates the dependence of the nutritive value on amino acid destruction. The shape of the curves and the inflexion point at the 80-minute period in both figures are convincing evidence. The necessary balance of essential amino acids is such that the elimination or the reduction below physiological needs of a single amino acid will result in impaired growth response. Here, the concentration of two amino acids is significantly lowered after only 15 minutes of autoclaving, while in 80 minutes destruction is virtually complete. In addition, two other amino acids are about 40 per cent destroyed.

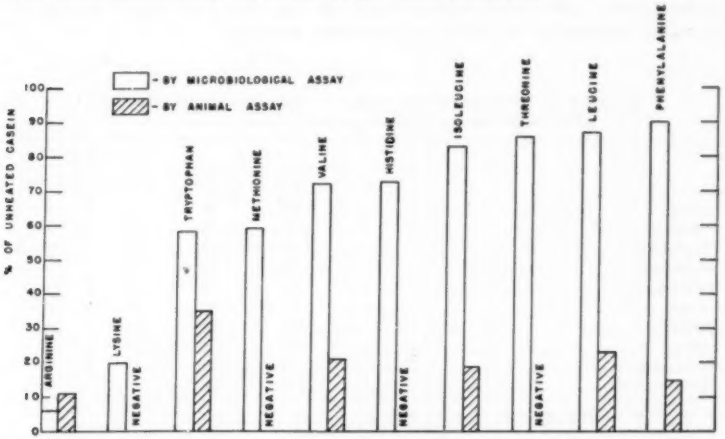

FIG. 3. Loss of amino acids in casein-dextrose heated 120 minutes at 15 p.s.i.

For these determinations the protein-dextrose complexes were acid-hydrolyzed (except for tryptophan) and the level of amino acids present was measured by their physiological availability for specific microorganisms. This does not necessarily mean that they will be available to the rat to the same extent. This has proved to be the case.

Casein-dextrose which had been heated for two hours at 15 p.s.i. was fed to rats in otherwise complete diets. To each diet was added nine essential amino acids at levels as they appear in unheated casein. In each diet one of the essential amino acids was omitted. The growth response was calculated after 28 days and compared with growth on a diet containing unheated casein. In Figure 3 are shown the results of this experiment (striped bars) compared with the results obtained by microbiological analysis (open bars). The values are expressed as a percentage of unheated casein.

In all cases except for arginine, the destruction was greater when measured by animal assay methods than when measured by microbiological methods. Threonine is the most striking in this difference. By animal assay this amino acid is almost completely unavailable, whereas by microbiological assay there was only 14 per cent destruction. Methionine and histidine

show similar discrepancies, while arginine and lysine show most similar results when assayed by the two methods. It is evident that one cannot depend too heavily on microbiological assays to demonstrate the complete story of amino acid nutrition. Animal assays with rats or other animals appears to be a necessity. Friedman and Kline (1950) have recently reported similar results with dextrose and protein hydrolysates.

The difference between the values obtained by the two methods may be due to the loss in "in vivo" digestibility. As yet we have been unable to check this point because of the difficulty of a quantitative collection of feces. The feces obtained from rats feeding on casein-dextrose diets is a sticky, tarry semi-liquid, which clings to the rats and cages, and it is virtually impossible to collect quantitative samples.

TABLE 2

Retention of Essential Amino Acids in Casein and Casein-Dextrose After 20 Minutes and 120 Minutes of Heating at 15 p.s.i.

	Casein		Casein-dextrose	
	20 min.	120 min.	20 min.	120 min.
Lysine	97[1]	80	62	20
Arginine	100	95	22	6
Tryptophan	100	94	92	58
Methionine	98	96	70	59
Histidine	97	83	73	73
Valine	95	95	91	72
Isoleucine	100	100	91	83
Leucine	100	100	95	87
Threonine	100	100	85	86
Phenylalanine	100	100	96	88

[1] Per cent of that found in unheated casein

Further evidence that the reducing sugar in the protein-sugar mixture during autoclaving is the cause for the loss in biological value of the protein is illustrated by comparisons of microbiological assays and animal assays of casein autoclaved with and without dextrose. The destruction of biological value caused by the presence of the dextrose over and above that caused by the heat alone is evident from the data shown in Figure 1 and Table 2. Although the microbiological assay data shown in the table are for only two heating periods, a range of from ten to 275 minutes was run.

The reaction that takes place when proteins are heated with dextrose is evidently that of the non-enzymatic browning (Maillard) reaction between the free amino groups of the protein and the carbohydrate. This can be shown by following the change in free amino groups of the protein and by measuring the amount of carbohydrate bound by the protein when casein is heated with dextrose at 15 p.s.i. for from 0 to 275 minutes.

The change in free amino groups was followed by measuring the change in the ability of the casein to bind a dye, Orange G. In Figure 4 it will be seen that the protein loses free amino groups (loss in dye binding capacity) very rapidly during the first few minutes of heating, and in about 80 minutes reaches a plateau.

This dye binding curve follows rather closely the biological value curve and the amino acid curves for arginine and lysine.

Infrared spectrophotometric work by our Organic Laboratory has shown that, when casein is heated with dextrose for two hours at 15 p.s.i., the amino groups of the original casein have been masked by masses of hydroxyl groups. Carboxyl groups remained unchanged. In addition, dye binding studies also demonstrated that there were no losses of carboxyl groups from the protein when casein was heated progressively with dextrose.

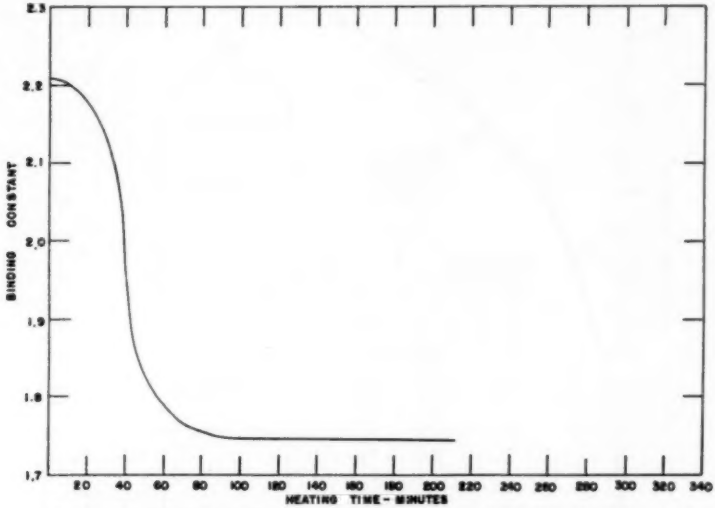

FIG. 4. Loss of amino groups in casein-dextrose heated at 15 p.s.i. Determined by dye-binding technique.

On the other hand, the amount of carbohydrate bound by the casein when heated from 0 to 275 minutes does not follow the biological value curve. The values for the carbohydrate bound follow a smooth curve as indicated in Figure 5. The values continue to rise past the 80-minute period, and at 275 minutes heating the protein continues to bind carbohydrate. The two factors, loss in amino groups and carbohydrate binding, when considered together, would indicate that the casein binds carbohydrate through reaction with amino groups up to the 80-minute period. Beyond this point there must be a condensation or "clumping" of carbohydrate around the carbohydrate already bound through amino groups. Similar data have been presented by Lea (1950) for casein and dextrose stored at 37°C.

That the change in digestibility also has some effect upon the change in biological value has been demonstrated in preliminary experiments with simultaneous "in vitro" enzymatic digestion-dialysis of protein-dextrose complexes followed by paper chromatography.

The apparatus consists of a dialysis bag hung in a cylindrical container immersed in a water bath as schematically shown in Figure 6. Appropriate buffer is placed both in the bag and outside of the bag in the container. The protein preparation to be digested and the appropriate enzymes are added to the bag. The contents of the bag are stirred continuously. Buffer is permitted to drip slowly into the bag and at the same rate as at which the dialyzed end products of the digestion are drawn off from the bottom of the container. At specified intervals samples are taken from the draw-off. Amino

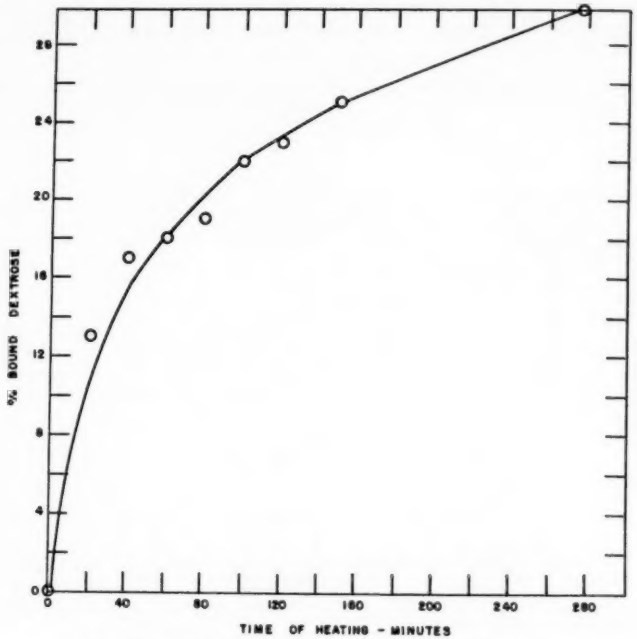

FIG. 5. Binding of dextrose by casein heated at 15 p.s.i.

nitrogen was determined on a portion of this sample by the method of Pope and Stevens (1939). Another portion was carefully evaporated to dryness at a low temperature, taken up in a small amount of ethyl alcohol, and subjected to paper partition chromatography according to the usual methods. Since it was found that peptides diffused through the dialysis bag, another portion of the dialysate was hydrolyzed with 5 N HCl and the above determinations again run on the digest after removal of excess HCl.

By this method it was found that the digest of casein-dextrose autoclaved for 40 minutes showed fewer spots than did unheated casein or unheated casein-dextrose. Certain of the amino acids showed up much later and in lower concentration in the dialysate from the heated casein-dextrose than they did from the other samples. This work is far from complete at this time, but it does indicate a difference in the rate of release of certain of the essential amino acids.

The effect of ranges of processing temperatures on the biological value of casein-dextrose was also determined. Casein-dextrose was autoclaved for

20 minutes and 120 minutes at 100°C.(212°F.) (0 p.s.i.), 109°C.(228°F.) (ten p.s.i.), 115.5°C.(240°F.) (ten p.s.i.), and 121°C.(250°F.) (15 p.s.i.). These temperature effects are shown in Figure 7. The important fact to be noted here is that raising the temperature on a 20-minute process has a greater effect than raising the temperature on a 120-minute processing time. This is probably due to the fact that considerable damage had already been effected in 120 minutes at the lower temperature, and further damage at the higher temperature was less likely to occur. On the other hand at the 20-minute period less damage had taken place, and when the material was processed at a higher temperature there was possibility of further impairment.

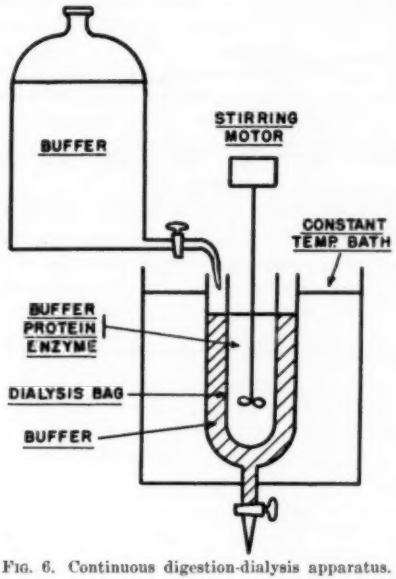

FIG. 6. Continuous digestion-dialysis apparatus.

The effect of the moisture level in the processed mixture was also investigated. Mixes were prepared of casein, dextrose, and water, with the moisture level varying from about one per cent to 68 per cent. This corresponds to mixtures of casein plus dextrose with no added water, to water in amounts of four ml. per gram of casein. The results obtained are shown in Figure 8. With no added water (about one per cent in the mix) there was extreme impairment in the nutritive value of the casein. As the moisture content was increased to 20 per cent, there was a small increase in the biological value which then remained constant up to 51 per cent moisture. When an increase was made to 68 per cent, there was an abrupt rise in the nutritive value of the protein. However, at this level the protein nutritive value was at bare maintenance level and in compari-

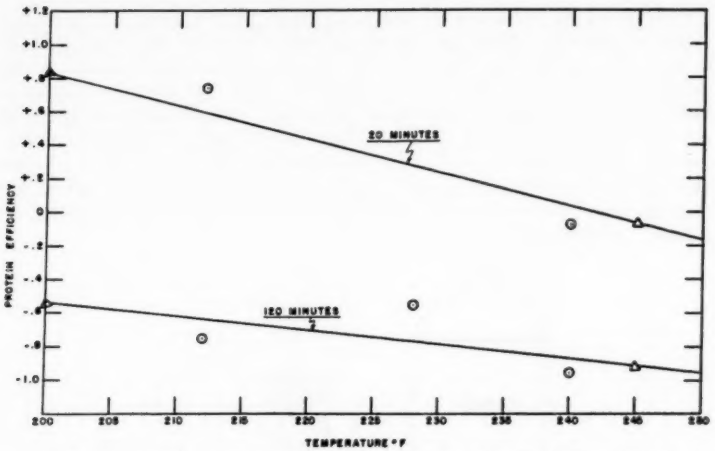

FIG. 7. Effect of temperature on biological value of casein-dextrose heated 20 minutes and 120 minutes.

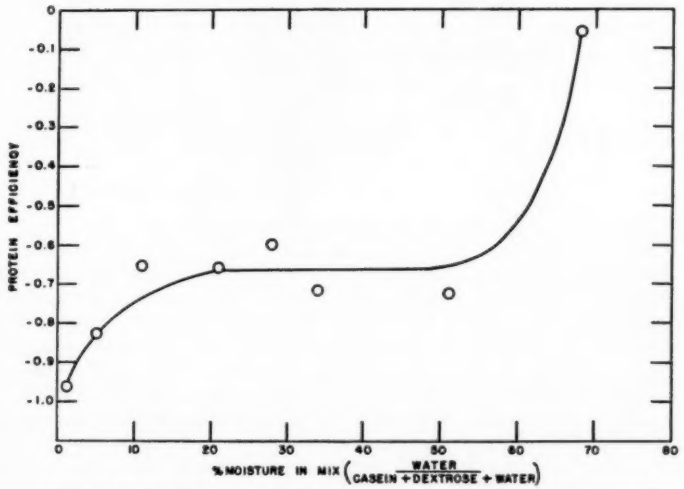

FIG. 8. Effect of moisture level on biological value of casein-dextrose. 120 minutes heating time at 15 p.s.i.

son with unheated casein was very poor. The consistency of the mixture at this level was a thin gruel with much free water.

SUMMARY

It has been demonstrated that the non-enzymatic browning reaction (Maillard reaction) is an important consideration from the standpoint of nutrition in the processing of food materials.

Autoclaving casein with dextrose results in a rapid loss in nutritive value of the protein. This decrease is over and above that caused by the heat alone. A greater portion of this loss is caused by the rapid destruction of some of the essential amino acids, particularly lysine and arginine, as shown by microbiological analyses. By animal assay methods, arginine, lysine, methionine, histidine, and threonine were found unavailable to the young rat for growth.

It has been shown that when casein is heated with dextrose in an autoclave at 121°C.(250°F.), up to about 80 minutes of heating, the protein binds carbohydrate through its free amino groups. After this time further carbohydrate is apparently bound by condensation around carbohydrate already bound by amino groups.

Increasing the water content of the casein-dextrose mixture during autoclaving inhibits the reaction to some extent, with a resulting increase in nutritive values. However at 68 per cent moisture the protein is of such quality that it will only barely maintain weight, and in comparison with unheated casein the nutritive value is very poor.

Increasing the temperature of processing from 100°C. to 121°C.(212°F. to 250°F.) affects the protein of the casein-dextrose adversely. Increasing the temperature of a short time process (20 minutes) has more adverse effect than increasing the temperature on a long time process (120 minutes).

There is some evidence to indicate that autoclaving casein with dextrose also decreases the digestibility of the protein, but this effect is minor compared with losses of essential amino acids.

Acknowledgment

We wish to acknowledge the expert assistance of Mr. W. J. Ohan, Mr. R. Waltz, Mr. K. W. Schwartz, and Mr. H. Welsch.

REFERENCES

FRAENKEL-CONRAT, H., AND COOPER, M., 1950. The use of dyes for the determination of acid and basic groups in proteins. J. Biol. Chem., **154**, 239-346.

FRIEDMAN, L., AND KLINE, O. L., 1950. The relation of the amino acid-sugar reaction to the nutritive value of protein hydrolysates. J. Nutrition, **40**, 295-307.

HORN, M. J., JONES, D. B., AND BLUM, A., 1946. Microbiological determination of methionine in proteins and foods. J. Biol. Chem., **166**, 321-326.

LEA, C. H., 1950. The role of amino acids in the deterioration of food: the browning reaction. Chem. Inds., 155-158.

LOWRY, J. R., AND THIESSEN, R., JR., 1950. Studies of the nutritive impairment of proteins heated with carbohydrates. II. In vitro digestion studies. Arch. Biochem., **25**, 148-156.

MCINROY, E. E., MUREE, H. K., AND THIESSEN, R., JR., 1949. The effect of autoclaving with dextrose on the nutritive value of casein. Arch. Biochem., **20**, 256-260.

POPE, C. G., AND STEVENS, M. F., 1939. The determination of amino nitrogen using a copper method. Biochem. J., **33**, 1070-1077.

GROWTH OF BACTERIA ASSOCIATED WITH FOOD POISONING EXPERIMENTALLY INOCULATED INTO DEHYDRATED MEAT [1]

MILTON SEGALOVE [2] AND G. M. DACK
Food Research Institute, The University of Chicago, Chicago, Illinois

[Received for publication, July 11, 1950]

The problems involved in the production and distribution of dehydrated meats have received attention recently under the Emergency Meat Dehydration Investigations, sponsored by the U. S. Department of Agriculture. The chemcial composition and nutritive value of dehydrated meat have been thoroughly investigated, Orent-Keiles, Hewston, and Butler (1946), Hoagland and Snider (1946), and Hankins, Ernst, and Kauffman (1946). Rehydration problems were studied by Hankins and Hetzer (1947), and packaging research was reported by Whitmore, Seligson, Kraybill, and Webb (1948).

Since dehydrated meat is not a sterile product, the possible danger of food poisoning following its consumption must be evaluated. The finished package of dehydrated meat may be subjected to various conditions altering the contents of the package either before or after the container is opened. The type of meat (beef, pork, etc.), concentration of salt, presence or absence of nitrates or nitrites, time and temperature of storage, changes in moisture content during storage, temperature during rehydration, length of rehydration period, and the possible effect of naturally occurring contaminants are all things to be considered. The food poisoning organisms of chief concern are *Clostridium botulinum*, staphylococci and possibly streptococci. The *Salmonella* group could play an important role if the finished product were subjected to contamination of rodents.

The following experiments were done to determine (a) the conditions of moisture and length of storage necessary for *C. botulinum* to elaborate its toxin, and (b) the conditions which would allow growth of staphylococci, streptococci, and salmonellae. The organisms used in these studies were *Clostridium botulinum*, Type A, *Staphylococcus aureus*, strain 161, alpha-type *Streptococcus*, and *Salmonella enteritidis*. The strains of staphylococci and streptococci used had been isolated from food poisoning outbreaks and had been shown repeatedly to cause food poisoning in appropriate laboratory animals and/or human volunteers during the course of previously conducted laboratory experiments. The strain of *S. enteritidis* used was isolated in 1914 from a patient who was caring for sick calves during an epizootic of enteritis.

The meat samples obtained for study were supplied by the U. S. Department of Agriculture, Bureau of Animal Industry, and some of the meat packers.

[1] This paper is the tenth in a series which deals with the Emergency Meat Dehydration Investigations of the United States Department of Agriculture and cooperators.

[2] Present address: Bio-Science Laboratories, Inc., Los Angeles, California.

MATERIALS AND METHODS

All samples of dehydrated meat with one exception were received in tin containers. The exception was a compressed sample wrapped in cellophane and received from a meat packer. Upon receipt, each sample was tested for original moisture and salt content and portions of the sample were adjusted to various moisture levels as required by the experiment.[3] In all cases the moisture was calculated on the total weight of the sample rather than on a fat-free basis.

Suspensions of organisms to be used for inoculation of the specimens were diluted in 15 ml. of a 4 per cent dextrose-2 per cent salt solution. This dextrose-saline solution has been found satisfactory for producing a uniform spray of droplets. The suspension was placed in a Glaseptic adrenalin vaporizer which was calibrated to deliver 0.51 ml. of the dextrose-saline suspension in one minute at an air pressure of 500 mm. of mercury. The meat specimens to be sprayed were spread out either in porcelain trays or petri dishes. The porcelain trays were used when spraying *C. botulinum* whereas petri dishes were used when spraying staphylococci, streptococci, and salmonellae. Approximately 100-gm. samples of meat were used in the botulinum experiments and 30-gm. samples were used with all others.

All spraying was done in a glass-enclosed cabinet which was placed within a glass-enclosed hood. After spraying, all meat samples were allowed to remain undisturbed for thirty minutes to allow for settling of the droplet particles. Upon removal from the spraying chamber the samples were transferred to sterile five-ounce tin cans and the lids secured with rubber bands.

The samples used for testing the growth of *C. botulinum* were not sterilized before inoculation since toxin production could be measured in the presence of other bacteria naturally present, whereas samples used for testing with staphylococci, streptococci, and salmonellae were autoclaved in airtight containers before inoculation.

EXPERIMENTS WITH CLOSTRIDIUM BOTULINUM

A potent toxin producing strain of *C. botulinum*, Type A was used. The spore suspension was prepared by growing the organisms in beef heart medium under a sterile vaseline seal for a period of ten days to two weeks. Following this incubation period the culture was shaken, transferred to centrifuge tubes and centrifugalized at low speed for three to five minutes. The supernatant containing the bacterial cells was then poured into another centrifuge tube and centrifugalized at high speed for one hour. The supernatant fluid was discarded and the sediment was washed twice with a volume of sterile distilled water equal to the original volume followed by a final washing with sterile saline. After each washing the cells were recovered by centrifugalizing for two hours at high speed. The resulting concentrated spore suspension was stored in sterile saline. Before the spore suspension was used, 0.5 ml. was removed aseptically from the storage bottle and put into a sterile test tube. This was heated at 80°C. for 30 minutes. The absence of toxin was repeatedly demonstrated in such heated suspensions. The number of viable spores was determined by making decimal dilutions in sterile saline and adding one ml. of each dilution to tubes of boiled beef heart medium. After inoculation the tubes were sealed with sterile vaseline and incubated at 33°C. for seven days. Growth was demonstrated by turbidity and gas production and was usually present in the $1:10^6$ dilution.

After the meat was sprayed with organisms and placed in tin containers, the containers were placed in a dry Pyrex desiccator jar, the jar was evacuated and then placed in a 37°C. incubator. Samples of meat were removed at varying intervals of time (Table 1). Before any sample was removed from the jar the vacuum was tested at the time of opening. A five-gm. portion of meat was removed from its container and placed in a sterile mortar with pestle. Twenty-five ml. of sterile saline were added, the sample was ground and most of the heavy particles allowed to settle. The supernatant fluid was poured into a sterile test tube and centrifuged for ten minutes at low speed to remove any remaining meat particles. The supernatant fluid was tested for the presence of botulinum toxin by intraperitoneal inoculation of one ml. of the fluid into white mice and by feeding five ml. of the same fluid by stomach tube to

[3] These determinations were carried out by the Research Laboratory of the American Meat Institute.

lightly anaesthetized guinea pigs. Ether was the anesthetic used. In the case of mice, control animals were inoculated with 0.25 ml. of homologous antitoxin mixed with the same volume of fluid as was inoculated into the test mice. Five mice, two of which were protected with antitoxin, were used in testing each sample. Three guinea pigs, one of which was protected with antitoxin, were used in each test.

TABLE 2

Range of Moisture, Salt, and Fat Content of Dehydrated Meat Samples Received for Testing

Kind of meat	Range of moisture content,	Range of salt content,	Range of fat content,
	pct.	pct.	pct.
Beef (27 samples)	1.37-12.8	0.17-10.78	8.34-41.40
Pork (23 samples)	2.17-19.9	0.10- 5.03	10.4 -35.0[1]
Ham (1 sample)	11.6	5.02	.....

[1] Approximately.

In a total of 51 samples of meat tested no toxin was detected in inoculated specimens adjusted to 10 and 20 per cent moisture and incubated at 37°C. for as long as nine months. These meat samples had a wide range of moisture, salt, and fat content (Table 2). In long term experiments six meat samples, including three beef and three pork specimens, were assayed for the presence of toxin. The results of these tests are summarized in Table 1. In no case was the presence of toxin detected in samples containing 30 per cent moisture or less after incubation for as long as nine months. However, when the moisture content was raised to 40 per cent, four samples were positive for toxin (one sample in one week and three in two weeks). All samples were positive for toxin in one week when the moisture content was raised to 60 per cent. In view of this latter result an experiment was done to determine the shortest time required for the production of botulinum toxin in samples of meat adjusted to 60 per cent moisture. The methods used were the same as previously described but the intervals of incubation before testing were 8, 12, 24, and 48 hours. In this experiment no toxin was demonstrated in the samples incubated for as long as 24 hours. The samples assayed for toxin after 48 hours' incubation were all positive. It was observed that no abnormal odor was present in samples which did not contain toxin. However, the 48-hour sample tested was obviously spoiled as evidenced by a putrefactive odor.

EXPERIMENTS WITH STAPHYLOCOCCI, STREPTOCOCCI AND SALMONELLAE

After the above organisms were grown in the appropriate mediums, suspensions were sprayed in the same manner as described above. After inoculation the meat specimens were put into sterile tin cans and incubated at 37°C. At intervals of time indicated in the tables (3, 4, and 5) the cans were removed from the incubator and one-gm. samples removed for test under aseptic conditions. Each sample taken was placed in a sterile mortar and nine ml. of sterile saline were added. The specimen was ground with a sterile pestle and then poured into a sterile tube. After settling, the supernatant fluid was used for counts of viable bacteria by making decimal dilutions in nine-ml. sterile saline blanks. One ml. of each dilution was used in making pour plates. Duplicate plates were made of each dilution. For the streptococci counts, 0.5 per cent sheep blood agar was used for plating, whereas counts of staphylococci and salmonellae were made in nutrient agar.

TABLE 1

Presence of Botulinum Toxin in Experimentally Inoculated Dehydrated Meat Samples Adjusted to 10, 20, 25, 30, 40, 50, and 60 Per Cent Moisture, Incubated and Examined at Various Intervals[1]

Sample	Moisture,	Salt,	Fat,	Presence of botulinum toxin in samples adjusted to various moisture levels[2]										
				40 pct.					50 pct.	60 pct.				
				1 wk.	2 wks.	4 wks.	8 wks.	12 wks.	1 wk.	8 hrs.	12 hrs.	24 hrs.	48 hrs.	1 wk.
	pct.	pct.	pct.											
Beef No. 11	4.58	0.46	28.5	+						—	—	—	+	+
Beef No. 3	7.62	3.50	25.75	—	—	—				—	—	—	+	+
Beef W	7.98	2.53	.....	—	+					—	—	—	+	+
Pork lot Y	8.80	0.47	29.59	—	+					—	—	—	+	+
Pork No. 39	7.77	1.73	.....	—	—	—	—	—	+	—	—	—	+	+
Pork lot B-395	11.00	2.64	31.10	—	+				+	—	—	—	+	+

[1] The 10, 20, 25, and 30 per cent moisture samples tested after 2, 4, 8, and 12 weeks and 6 and 9 months were all negative.
[2] The sign + indicates toxin agent as evidenced by death of mice and guinea pigs, the sign —, no toxin present.

Staphylococci. In the case of specimens inoculated with a food-poisoning strain of staphylococci, no growth occurred when the moisture content of the meat samples was 10 to 20 per cent. In both cases there was death of the organisms during the incubation period. At 30 per cent moisture, growth occurred in all pork samples except the one with a salt concentration of 4.56 per cent. However, in the beef samples, no growth occurred except for a slight increase in the sample with a salt concentration of 2.20 per cent. At 40, 50, and 60 per cent moisture, growth occurred in all pork and beef samples regardless of salt content. In the case of pork, increased salt content seemed to retard growth. In the case of beef, the inhibitory effect of salt was not evident; in fact, higher counts were obtained with samples of highest salt content (Table 3).

Streptococci. Where a strain of streptococci isolated from a food poisoning outbreak had been similarly inoculated into beef and pork samples of specified salt and moisture concentrations, there was no growth at 10, 20, or 30 per cent moisture with the exception of two pork samples which showed slight growth, one after 48 hours and the other after one week. At 40 per cent moisture, growth occurred in two pork samples containing low salt concentrations, whereas slight growth was evident in pork samples with the next highest salt concentration, and no growth was evident in the highest salt concentration (0.23, 1.94 and 3.09, 4.56 per cent respectively). Thus, salt seemed to have a definite inhibitory effect upon growth. In beef adjusted to 40 per cent moisture, slight growth occurred at the lowest salt concentration but not in the three samples with higher salt concentrations. At 50 and 60 per cent moisture, growth occurred in all pork and beef samples. However, the inhibitory effect of salt on growth was evident in both cases (Table 4).

Salmonellae. In pork and beef samples adjusted to 10, 20, 30, 40, and 50 per cent moisture no growth occurred with the strain of *Salmonella* used during the test period studied. At 60 per cent moisture, growth occurred in all samples except the two beef with the two highest salt concentrations and one pork sample which contained the highest salt content (Table 5).

SUMMARY AND CONCLUSIONS

Experiments were carried out to determine the moisture content and length of incubation necessary for *C. botulinum*, Type A, and for food poisoning strains of staphylococci, streptococci, and salmonellae to grow.

It was found that *C. botulinum* will grow and produce its toxin in 48 hours but not in 24 hours in meat samples containing 60 per cent moisture and incubated at 37°C. In samples containing 40 per cent moisture, toxin could be detected in one week after incubation at 37°C. Samples containing 30 per cent moisture or less showed no evidence of toxin even after incubation at 37°C. for as long as nine months.

Growth of food poisoning strains of staphylococci or streptococci isolated from food poisoning outbreaks was not evident in samples containing 20 per cent moisture or less. When a moisture content of 30 per cent was reached, growth of the organisms was dependent upon the salt content of the sample. High salt content inhibited growth. The *Staphylococcus* strain

TABLE 3

Numbers × 10^6 of Viable Staphylococci in Experimentally Inoculated Meat Adjusted to 10, 20, 30, 40, 50, and 60 Per Cent Moisture, Incubated and Examined at Intervals of from 4 Hours to 2 Weeks

Samples	Salt content,[1] pct.	10 pct.			20 pct.			30 pct.				40 pct.				50 pct.				60 pct.			
		Inoc.	1 wk.	2 wks.	Inoc.	1 wk.	2 wks.	Inoc.	24 hrs.	48 hrs.	1 wk.	Inoc.	8 hrs.	12 hrs.	48 hrs.	Inoc.	8 hrs.	12 hrs.	24 hrs.	Inoc.	4 hrs.	8 hrs.	12 hrs.
Pork No. 393	0.23	.07	.01	.0009	.06	.016	.006	.03	164	1470	....	.03	3.4	470	....	.04	78	1670	6100	.02	.35	103	950
Pork No. 394	1.94	.05	.0003	.00001	.09	.0004	0	.03	2.9	1640	....	.05	1.3	41	....	.04	41	1590	....	.04	.29	185	2040
Pork No. 395	3.09	.07	.003	0	.09	.07	0	.08	.05	.06	158	.06	.09	2.05	....	.04	1.67	173	5500	.06	.14	50	1020
Pork No. 375	4.56	.04	0	0	.07	0	....	.06	.05	.03	0	.11	.07	.07	2660	.07	.08	.26	1170	.09	.10	.45	43
Beef No. 410	0.21	.05	0	....	.05	.003	0	.06	.06	.03	.001	.10	.06	.11	1.04	.11	.23	2.9	810	.07	.21	4.3	124
Beef No. 409	2.20	....	....	....	.04	.002	0	.04	.04	.04	.05	.03	.05	.05	5.6	.03	.46	26	2410	.03	.06	3.7	340
Beef No. 408	3.09	.09	0	....	.06	.001	0	.06	.02	.03	.002	.06	.05	.05	500	.06	1.14	60	4500	.08	.21	34	460
Beef No. 413	4.17	.03	0	....	.05	.003	0	.04	....	.05	.008	.06	.11	.14	660	.05	1.2	162	5000	.09	.13	58	2140

[1] All salt given as original salt content of dehydrated sample before hydration.

TABLE 4

Numbers × 10^6 of Viable Streptococci in Experimentally Inoculated Dehydrated Meat Samples Adjusted to 10, 20, 30, 40, 50, and 60 Per Cent Moisture, Incubated and Examined at Various Intervals of Time

Samples	Salt content,[1] pct.	10 pct.			20 pct.			30 pct.			40 pct.				50 pct.				60 pct.			
		Inoc.	1 wk.	2 wks.	Inoc.	1 wk.	2 wks.	Inoc.	48 hrs.	1 wk.	Inoc.	12 hrs.	48 hrs.		Inoc.	8 hrs.	12 hrs.	24 hrs.	Inoc.	4 hrs.	8 hrs.	12 hrs.
Pork No. 393	0.23	.005	.002	0	.006	.002	0	.03	.03	.63	.04	1.4	....		.08	5.2	76	300	.05	.86	56	250
Pork No. 394	1.94	.007	.001	0	.006	.002	0	.03	.06	.003	.05	.03	125		.03	.48	4.8	27	.02	.31	18.6	94
Pork No. 395	3.09	.01	.002	0	.03	.008	0	.03	.03	.009	.02	.06	.05		.01	.06	.39	55	.02	.06	.03	.61
Pork No. 375	4.56	.007	.0002	0	.009	.00007	0	.02	.003	.0007	.08	....	.006		.009	.006	.009	13.5	.009	.01	.009	.18
Beef No. 410	0.21	.03	.0001	0	.05	.0003	0	.03	.02	.0003	.04	.07	.07		.04	.16	.18	25	.04	.10	1.0	30
Beef No. 409	2.20	....	....	....	.05	.0009	0	.06	.02	.003	.06	.04	.01		.04	.14	1.0	5.1	.05	.09	1.3	15
Beef No. 408	3.09	.005	.0006	0	.01	.0004	0	.08	.002	.0001	.02	.008	.005		.01	.02	.03	4.5	.01	.02	.24	8.2
Beef No. 413	4.17	.01	.005	0	.01	.004	0	.02	.012	.001	.02	.01	.01		.02	.04	.05	.08	.02	.03	.16	1.8

[1] Original salt content of dehydrated sample before hydration

TABLE 5

Numbers × 10^6 of Viable Salmonellae in Experimentally Inoculated Dehydrated Meat Samples Adjusted to 10, 20, 30, 40, 50, and 60 Per Cent Moisture, Incubated and Examined at Various Intervals of Time

Samples	Salt content,[1] pct.	10 pct.		20 pct.		30 pct.		40 pct.			50 pct.			60 pct.		
		Inoc.	1 wk.	Inoc.	1 wk.	Inoc.	1 wk.	Inoc.	4 hrs.	24 hrs.	Inoc.	12 hrs.	24 hrs.	Inoc.	8 hrs.	12 hrs.
Pork No. 659a	0.28	.006	0	.007	0	.006	0	.01	.003	0	.009	.0008	.02	.02	.005	.3
Pork No. 659b	1.02	.01	0	.01	0	.01	0	.01	.004	0	.01	.0006	.001	.02	.06	.4
Pork No. 659c	1.96	.006	0	.006	0	.009	0	.01	.0002	0	.02	.0008	.0004	.008	.01	.3
Pork No. 659d	3.51	.008	0	.007	0	.009	0	.01	.0001	0	.009	.0005	.0002	.009	.004	.002
Beef No. 668a	0.28	.01	0	.007	0	.007	0	.01	.0007	0	.03	.001	.0007	.01	.02	.05
Beef No. 668b	1.00	.003	0	.003	0	.003	0	.004	.0003	.00002	.007	.0007	.0002	.009	.006	.07
Beef No. 668c	2.20	.004	0	.006	0	.004	0	.007	.0002	0	.01	.0005	.0001	.02	.01	.01
Beef No. 668d	3.70	.003	0	.005	0	.005	0	.004	.0001	0	.009	.0003	.00009	.01	.004	.002

[1] Original salt content of dehydrated sample before hydration.

used grew in all meat samples containing 40 per cent or more moisture regardless of salt content. The streptococci grew feebly only in the beef sample with the lowest salt content adjusted to 40 per cent moisture and grew in three pork samples of low salt content containing the same amount of moisture. However, at 50 and 60 per cent moisture, growth occurred in all beef and pork samples.

The *Salmonella* strain used grew scantily in only one pork sample at the lowest salt concentration adjusted to 50 per cent moisture. In meat samples adjusted to 60 per cent moisture, growth of *Salmonella* occurred in all but three samples of high salt content.

REFERENCES

HANKINS, O. G., ERNST, A. J., AND KAUFFMAN, W. R., 1946. Chemical composition of raw and dehydrated meat. Food Research, 11, 501-506.

———, AND HETZER, H. B., 1947. Rehydration of dehydrated meat. Food Research, 12, 111-117.

HOAGLAND, R., AND SNIDER, G. G., 1946. Nutritive value of protein in dehydrated meat. Food Research, 11, 494-500.

ORENT-KEILES, E., HEWSTON, E. M., AND BUTLER, L., 1946. Effects of different methods of dehydration in vitamin and mineral values of meats. Food Research, 11, 486-493.

WHITMORE, R. A., SELIGSON, D., KRAYBILL, H. R., AND WEBB, B. H. Packaging dehydrated meat. Food Research, 13, 19-28.

A STUDY OF METHODS OF PREPARING FOOD PRODUCTS FOR MICROBIOLOGICAL ANALYSES[1]

A. H. JONES AND W. E. FERGUSON

[Received for publication, July 11, 1950]

The method of preparing food products for analysis is of considerable importance, since the resulting counts of microorganisms obtained depend to a large extent on the effectiveness of mixing and agitation in freeing the organisms from the surface. The usual method of preparing products for analysis usually consists of either volumetric measuring or weighing of the product and diluting with a definite quantity of sterile water. This is followed by a shaking procedure, usually for a definite period of time, either by hand or mechanical shaker, and further dilutions are then made to provide colony counts between 30 and 300.

In recent years the Waring Blendor has made its appearance and its use has been advocated for the preparation of products for analysis, Committee on Microbiological Examination of Foods (1946); Fabian and Orloff (1950); Goresline (1948); Van Eseltine, Nellis, Lee, and Hucker (1948). While this machine is effective in reducing a food product to a homogeneous mass, certain problems have arisen resulting from its use which led to the present study to determine differences in counts in foods, hand or mechanically shaken, as compared with counts from products comminuted in the Waring Blendor.

During the war years this laboratory made considerable use of the Waring Blendor for comminuting dehydrated products for moisture determinations. The dry product was subjected to comminution to a point where it was considered homogeneous. During this process of blending, the temperature increased from approximately 25°C. to 55°C. in a period of five minutes. In ten minutes, the time usually employed, the temperature had increased to 75°C. Bacteriological analyses of products so comminuted showed that bacteria in the products had almost disappeared. Inoculations were made in duplicate on tryptone-glucose agar and plates were incubated at 20°C. to 25°C. for four days. Counts obtained from the same product prepared by weighing a portion of the product into sterile jars and rehydrating for two hours in sterile water at room temperature followed by shaking by hand frequently, showed well over 100,000 per gram. This indicated that treatment of the dry product in the Waring Blendor resulted in markedly reduced bacteria counts. For this reason, the use of the Waring Blendor for comminuting dehydrated products in the dry state was discontinued.

A present study of methods for preparing for analyses products such as dehydrated, frozen fruits and vegetables, and gelatin has been carried out at various intervals. In these tests, counts were obtained on samples

[1] Contribution No. 306 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

of the same product shaken by hand, by mechanical shaker, or comminuted in the Waring Blendor.

EXPERIMENTAL

Gelatin Powder. Analyses of different one-gram portions of the same sample of gelatin mixed for two minutes in the dry state in the Waring Blendor show that bacteria counts are considerably reduced when compared with counts of the same sample of gelatin analyzed without such treatment. The range of counts for the untreated portions of gelatin was 5,000 to 10,800 with an arithmetic average of 8,800; portions of gelatin blended for two minutes showed a range of 300 to 1,800 with an arithmetic average of 650. During the treatment the temperature of the gelatin increased from 33°C. to 56°C. in two minutes. This may have been partly responsible for the reduction in count. The effect of increased temperature will be discussed in a later portion of this paper.

Frozen and Dehydrated Fruits and Vegetables. The results of analyses of 19 samples of miscellaneous dehydrated and frozen vegetables and fruits prepared both by shaking by hand and by comminution in the Waring Blendor are presented in Table 1.

TABLE 1

Comparison of Counts on Various Dilutions of Products Blended and Hand-Shaken Before Plating

Product	Bacteria count per gram[1]		Blended count as per cent of hand-shaken
	Hand-shaken	Blended	
Dehydrated potato	4,400	20,000	455
Dehydrated potato	12,500	38,500	308
Dehydrated potato	75,000	156,000	208
Dehydrated potato	16,000	13,000	81
Dehydrated potato	14,500	23,000	159
Dehydrated potato	1,700	17,000	1,000
Frozen spinach	200	1,000	500
Frozen spinach	3,000	4,000	133
Frozen peas	13,000	18,000	138
Frozen peas	16,500	14,500	88
Frozen cauliflower	8,500	11,000	129
Frozen cauliflower	12,000	18,000	150
Frozen blueberries	27,500	58,000	211
Frozen blueberries	20,000	10,000	50
Frozen asparagus	64,000	220,000	344
Frozen asparagus	7,500	10,500	140
Frozen wax beans	1,000	231,000	23,100
Frozen wax beans	11,000	5,500	50
Frozen green beans	165,000	1,200,000	727

[1] Samples shaken or blended for two minutes.

To all products in this series, treated in the Waring Blendor, sterile water had first been added; with dehydrated products the 1/20 dilution and with frozen products the 1/2.5 dilution was used. The results show considerable variation. Of the 19 samples, nine showed definitely higher counts when the product was comminuted in the Waring Blendor. Four samples were higher with the usual hand-shaking procedure. Six samples showed no important difference in counts between the two methods. Mention should be made of the great difference in bacteria count of one product (231,000 per gram) between portions comminuted in the Waring Blendor and those prepared by the hand-shaking method. The product treated in the Waring Blendor, in this instance, showed a count 231 times greater than the count of the product shaken by hand. Van Eseltine, *et al.*, also found one sample out of line in their analyses of frozen peas comminuted in the Waring Blendor. Other than questioning this greatly increased count in the product disintegrated in the Waring Blendor, no mention is made of the probable reason.

More extensive studies were made with dilutions of fresh beans comparing the hand and mechanically shaking methods with comminution in the Waring Blendor.

Periods of shaking or comminution in the first series were from 30 seconds and, at intervals of one minute, up to ten minutes. The range of counts in this series (Table 2),

TABLE 2

The Effect of Shaking by Hand or Machine, or Using the Waring Blendor on Bacteria Counts in Fresh Beans (Series 1)

Period of mixing	Bacteria count per gram in thousands[1]			Product temperature in Waring Blendor, (°C.)
	Hand-shaking	Machine-shaking	Waring Blendor	
0	8	....	....	33.0
30 sec	49	32	165	33.5
1 min	16	32	185	35.0
2 min	49	36	33	37.5
3 min	20	43	135	39.5
4 min	25	30	125	41.0
5 min	37	21	575	42.5
6 min	21	17	18	44.0
7 min	37	24	25	46.0
8 min	52	63	2	47.5
9 min	48	57	18	48.5
10 min	223	65	7	50.0
Arithmetic average	52	38	117	....
Logarithmic average	39	35	44	....
Range	16-223	17-65	2-575	....

[1]Averages of duplicate samples.

using shaking by hand, was from 16,000 to 223,000, with an arithmetic average over the entire ten-minute period of 52,000 per gram and a logarithmic average of 39,000 per gram. The range of counts in products shaken by mechanical shaker was 17,000 to 65,000, with an arithmetic average of 38,000 per gram and a logarithmic average of 35,000 per gram. Counts obtained with the Waring Blendor method ranged from 2,000 to 575,000 with an arithmetic average of 117,000 per gram and a logarithmic average of 44,000 per gram. However, after seven minutes' treatment in the Waring Blendor, there was a marked decrease in numbers of organisms, possibly due to the heating of the product. The temperature increased from 33°C. to 42.5°C. in seven minutes and after ten minutes was 50°C. Considering only the counts before the seven-minute period, the range was 33,000 to 575,000 with an average of 203,000. From this series it is apparent that greater variations in the bacterial count of products may occur when the Waring Blendor is used to comminute products prior to analysis. The results also indicate that there is no advantage in continuing shaking longer than two minutes, the usual period employed, since no important increase in count was noted following this period.

Results from a second series of tests on beans (Table 3), comparing the three methods, using intervals of 30 seconds, one minute, and two minutes, were practically the same with all three. In this series the initial count of the product was very low compared with that of the first series. The two-minute interval for each method with five replicate samples of product showed a range of counts for the hand-shaking method from 1,200 to 2,800 with an average of 2,000; the product mechanically shaken showed a range from 1,400 to 2,700 with an average of 2,000. The Waring Blendor method again showed the greatest variation with counts from 1,400 to 4,000 although the average of 2,200 was similar to those of the two other methods.

Pure Culture Studies. The effect of comminution of products in the Waring Blendor on the bacterial count was further studied using pure cultures of bacteria in different substrates. Included in the organisms studied were two species of micrococci, one of *Flavobacterium*, a culture of *Chromobacterium violaceum*, one of *Sarcina lutea* and one of *Serratia marcescens*. Water, milk, and an extract prepared from beans were compared

as dilution substrates. The initial suspension of the test organism, prepared from agar slopes, was inoculated into carefully weighed sterile substrate dilution blanks. A series of each substrate was shaken in a mechanical, horizontal reciprocating shaker;[2] the other was blended in the Waring Blendor for periods of 30 seconds, one, two, and five minutes.

The results obtained (Table 4) indicate that the effect of treatment of the suspensions in the Waring Blendor varies with different species of bacteria. In this table and in Table 5 the machine-shaken counts have been taken as 100 and the percentage increase or decrease has been calculated therefrom. In other words, a percentage higher than 100 indicates that the Waring Blendor count was greater than the machine-shaken series. Percentages lower than 100 indicate that the Waring Blendor counts were reduced as compared with machine-shaken suspensions.

TABLE 3

The Effect of Shaking by Hand or Machine, or Using the Waring Blendor on Bacteria Counts in Fresh Beans, Determined on Five Portions of the Mixtures (Series 2)

Portion No.	Bacteria count per gram								
	Hand-shaking			Machine-shaking			Waring Blendor		
	30 sec.	1 min.	2 min.	30 sec.	1 min.	2 min.	30 sec.	1 min.	2 min.
1	1,000	1,000	2,600	1,600	1,200	2,700	1,700	3,900	1,700
2	1,100	700	1,900	1,300	700	2,200	1,800	7,600	4,000
3	700	1,600	2,800	800	1,200	1,800	1,300	700	2,000
4	1,100	900	2,300	800	900	1,900	260	1,500	1,800
5	2,000	1,100	1,200	1,100	1,400	1,400	2,700	2,900	1,400
Arithmetic average	1,200	1,100	2,200	1,100	1,100	2,000	1,600	3,300	2,200
Logarithmic average	1,100	1,000	2,100	1,100	1,000	1,800	1,200	2,500	2,000
Range	700-2,000	700-1,600	1,200-2,800	800-1,600	700-1,400	1,400-2,700	260-2,700	700-7,600	1,400-4,000

Although considerable variability may be observed from the results presented, there is a general trend in most instances. With the two micrococcus species, there was very little difference in the counts between the different substrates and the two methods of mixing. With the first species of micrococcus noted in this table, 11 counts at the different periods with the Waring Blendor were either higher or similar to the suspensions mechanically shaken. One count (five minutes, water series) was significantly lower. The second species of micrococcus showed a similar picture with nine Waring Blendor counts being either higher or very close to the machine-shaken. Three counts were definitely lower.

The one species of *Flavobacterium* suspended in water showed decreased Waring Blendor counts with the longer periods of mixing. With the exception of one or two counts, results with this organism showed substantially reduced counts when the Waring Blendor was used.

Using water as a substrate with *Chromobacterium violaceum*, the counts with the Waring Blendor were greatly reduced; in fact, after five minutes' comminution in the Waring Blendor, this organism had practically disappeared. With milk and vegetable extract, the counts were generally higher when the Waring Blendor was used.

Sarcina lutea, the one organism which showed definite microscopical evidence of clumping, gave increased counts in all substrates at different periods when the Waring Blendor was used.

Perhaps of greater significance are the average counts of the six organisms mixed by shaking in the mechanical shaker or comminuted in the Waring Blendor (Table 5). It is evident from the data presented that milk provided more protection for bacteria

[2] Manufactured by the Precision Scientific Company.

TABLE 4

The Effect of Shaking by Machine, or Using the Waring Blendor on the Counts From Pure Cultures of Microorganisms in Different Substrates

Test organism	Period of mixing	Water			Milk			Bean extract		
		Bacteria count per gram in thousands			Bacteria count per gram in thousands			Bacteria count per gram in thousands		
		Machine	Waring Blendor	Pct.[1]	Machine	Waring Blendor	Pct.[1]	Machine	Waring Blendor	Pct.[1]
Micrococcus sp.	30 sec.	96	126	131	221	239	108	237	217	92
	1 min.	82	79	96	198	230	116	189	285	151
	2 min.	80	90	113	214	268	125	170	224	132
	5 min.	113	52	46	184	260	141	182	187	103
Micrococcus sp.	30 sec.	510	520	102	490	440	90	420	211	50
	1 min.	410	390	95	450	380	84	232	138	59
	2 min.	360	390	108	440	370	84	231	386	167
	5 min.	420	370	88	173	306	177	320	219	68
Flavobacterium sp.	30 sec.	340	650	191	1,080	840	78	1,370	810	59
	1 min.	500	300	60	1,250	790	63	1,280	940	73
	2 min.	910	350	38	1,300	550	42	1,260	940	73
	5 min.	410	174	42	720	650	90	1,280	660	51
Chromobacterium violaceum	30 sec.	175	84	48	1,540	1,530	99	1,070	....	....
	1 min.	365	38	10	1,380	1,550	112	1,310	910	69
	2 min.	130	31	24	1,240	1,820	146	650	920	142
	5 min.	117	1	1	1,060	1,470	139	830	1,210	146
Sarcina lutea	30 sec.	8	14	175	9	13	144	13	15	115
	1 mir..	8	17	213	11	12	109	14	22	157
	2 min.	9	18	200	7	16	229	13	24	185
	5 min.	8	21	263	10	15	150	12	26	217

[1] Count obtained by use of Blendor as per cent of count obtained by machine shaking.

TABLE 5

Comparison of Average Counts of Six Pure Cultures of Bacteria Mixed in Machine Shaker or in the Waring Blendor

Substrate	Period of mixing	Bacteria counts per gram in thousands				Pct.[1]
		Machine shaker		Waring Blendor		
		Average counts	Average	Average counts	Average	
Water	30 sec.	405	419	422	257	61
	1 min.	459		277		
	2 min.	441		207		
	5 min.	371		108		
Milk	30 sec.	843	776	800	753	97
	1 min.	817		744		
	2 min.	814		799		
	5 min.	629		670		
Bean extract	30 sec.	875	763	511	580	76
	1 min.	780		599		
	2 min.	682		614		
	5 min.	712		595		

[1] Count obtained by use of Blendor as per cent of count obtained by machine shaking.

against the action of the Waring Blendor. The results using water or vegetable extract indicate that the Waring Blendor was responsible for decreased counts.

DISCUSSION AND CONCLUSIONS

It is not the purpose of this paper to condemn the use of the Waring Blendor for the preparation of foods for bacteriological analysis but rather to give actual results obtained in our analyses. Although the use of the Waring Blendor method for the preparation of food products for bacteriological analyses is widespread, the literature contains little published work to support its use. We have found that the Waring Blendor method requires more time for analysis since the Blendor must be first sterilized with an agent such as chlorine. This must be followed by treatment with a sterile solution of sodium thiosulphate to remove the chlorine. Such treatments require considerable time. On the other hand, comminution in the Waring Blendor does reduce most products to a homogeneous mass within two minutes. Sometimes the product, in the process of comminuting in the Waring Blendor, is reduced to so fine a state that difficulty is experienced in pipetting a portion of the product for analysis.

Our studies have brought out several important points insofar as the use of the Waring Blendor for comminution of food products is concerned. First, there is little doubt that in the analyses of dehydrated products such as fruits, vegetables, and gelatin powder which were mixed in the dry state, the bacterial content is thereby reduced substantially. Second, in the analysis of a number of miscellaneous samples of food products, 47 per cent of the samples gave higher counts, one abnormally high, and 21 per cent of the samples gave lower counts with the Waring Blendor when compared with the counts of hand-shaken samples. Thirty-two per cent of the samples showed no important difference between the two methods. Third, when more extensive studies were made with one product, it was found that this product, when comminuted in the Waring Blendor, showed a significantly higher average count. Enormous variations were also noted when these results were compared with results of the hand- or machine-shaken series. Fourth, products with high bacteria counts showed greater variations using the Waring Blendor than low count products, when compared with hand- or machine-shaken series.

Pure culture studies, using three different substrates with the Waring Blendor and shaking machine for mixing, showed that the counts varied with the organism used. The organism *(Sarcina lutea)* showed definite evidence of clumping by microscopical examination, and substantially increased counts were obtained when this material was comminuted in the Waring Blendor. Results with other test organisms varied with the substrate, some giving higher counts, others lower.

The overall picture suggests that either increased or decreased counts may be obtained as a result of comminuting the product in the Waring Blendor as compared with counts obtained either by hand or machine shaking of dilutions of samples of products. Whether or not the increased counts resulting from comminution in the Waring Blendor are desirable is debatable. Apparently the occurrence of such increased counts depends entirely on the type of organism present; an organism that produces clumps

may show greatly increased counts due to the fact that the Blendor breaks up these aggregations of organisms. Thus, instead of a colony resulting from a large aggregation of organisms, such as might be anticipated with hand or machine shaking, a colony from products comminuted in the Waring Blendor may result from smaller aggregations or from single organisms. Reduced counts, resulting from the use of the Waring Blendor, may be due to the increase in temperature, possibly a factor with products comminuted in the dry state, or to the grinding action which apparently is involved in the Waring Blendor process. It does not appear logical that the increase in temperature would be the only factor involved since suspensions of organisms have been placed in the Waring Blendor at a temperature of approximately 4.5°C. Under such conditions greatly reduced counts were obtained without any appreciable rise in temperature.

If the use of the Waring Blendor were adopted as a standard procedure, it would necessitate a revision of existing bacteriological standards for food products. Where abnormally high counts were obtained in a particular product it would be also necessary to ascertain the types of organisms involved.

REFERENCES

Committees on Microbiological Examination of Foods, 1946. Microbiological examination of foods. Tentative methods for the microbiological examination of frozen foods. Am. J. of Pub. Health, **36**, 322-334.

FABIAN, F. W., AND ORLOFF, M. D., 1950. Germicidal detergents are not needed for washing fresh-picked cucumbers. Food Inds., **22**, 256-259, 378, 380.

GORESLINE, H. E., 1948. Report on microbiological methods for frozen fruits and vegetables. J. Assoc. Offic. Agr. Chemists, **31**, 519-521.

VAN ESELTINE, W. P., NELLIS, L. E., LEE, F. A., AND HUCKER, G. J., 1948. Effect of rate of freezing on bacterial content of frozen vegetables. Food Research, **13**, 271-280.

THE NUMBERS AND TYPES OF BACTERIA FOUND ON THE HANDS OF FOOD HANDLERS

MURRAY P. HORWOOD AND VIRGIL A. MINCH
Department of Civil and Sanitary Engineering,
Massachusetts Institute of Technology,
Cambridge, Massachusetts

[Received for publication, July 21, 1950]

The public eating establishment has become an increasingly important factor in public health. Shifting populations, increasing numbers of people in industry, changing living habits, and increased travel have been factors influencing the change in the eating habits of the public. The number of food establishments in the United States exceeds 160,000 and includes restaurants, cafeterias, ice cream parlors, and lunch rooms. In addition there are more than 80,000 drinking establishments that also serve meals; about 50,000 bars, taverns, and clubs which do not serve meals; and nearly 40,000 drug stores with soda fountains.

Besides the innumerable persons fed daily in school cafeterias and dining rooms, boarding houses, industrial cafeterias, planes and trains, and hospitals and other institutions, it has been estimated that seven million persons eat at least one meal per day outside the home; and that more than twenty billion meals are eaten annually in the various public eating establishments.

This study deals with the collection and bacteriological examination of hand-washed samples derived from the hands of food handlers selected at random from 22 public eating establishments in Boston and Cambridge, Massachusetts.

METHODS

Food handlers were requested to wash both hands in 50 ml. of sterile buffered distilled water for one minute without the use of soap or other detergents but with the aid of rubbing.

The buffered distilled water was prepared according to the directions of the subcommittee on Food Utensil Sanitation of the Committee on Research and Standards of the American Public Health Association published in October, 1947. This is done by dissolving 34 gm. of KH_2PO_4 in 500 ml. of distilled water, adding 175 ml. of *N* NaOH solution, diluting to one liter with distilled water, adjusting this solution to pH 7.2, and diluting 1 ml. of this stock solution to 800 ml. with boiled and cooled distilled water. A measured amount of this phosphate buffer solution was placed in Pyrex test tubes (200 x 25 mm.) so that, after sterilizing in the autoclave at 15 p.s.i. (121°C.) for 20 minutes, 50 ml. remained in each test tube.

For the collection of each sample, the sterile buffer solution from one of these tubes was poured into a chemically clean, sterile Pyrex evaporating dish, 125 mm. in diameter and 65 mm. in depth. The food handler was instructed to wash the fingers of both hands thoroughly in the sterile buffer solution, including the area between the fingers and under the nails; and to rub each hand vigorously with the other during the process. A mechanical timer was used to limit the hand-washing procedure to exactly one minute. The food handlers were taken directly from their work. They did not have an opportunity to wash their hands before collecting the sample.

After collection, the sample was returned aseptically into the original tube, carefully stoppered with a rubber stopper, and taken to the laboratory for the bacteriological examinations. All samples were examined within four hours of the time of

collection and were kept under refrigeration in the laboratory until used. All samples were collected between July 6 and August 12, 1949.

The bacteriological examination included total plate counts at 20°C. and at 37°C., and tests for aerobic spore-forming bacteria, the coliforms and their differentiation, and the hemolytic cocci and enteric bacteria.

RESULTS

Total Counts. The total counts obtained from 34 hand-washing samples obtained in 22 establishments are given in Table 1. Fourteen samples were obtained from females and twenty from males. The samples were derived from cafeterias, lunch rooms, drug stores, restaurants, and four Chinese establishments. Hot foods as well as sandwiches and desserts were served in every instance.

TABLE 1

Distrubution of Total Count per ml. From Hand-Washed Samples After 48 Hours' Incubation on Tryptone Glucose Agar

Total count range	Incubation at 20°C.	Incubation at 37°C.
Under 1,000 per ml	1	0
1,000- 5,000	9	0
5,000- 10,000	5	3
10,000- 25,000	4	6
25,000- 50,000	2	2
50,000-100,000	6	6
Over 100,000	7	7
Total	34	34

The range in total count at 37°C. varied from 6,200 to 16,000,000 per ml. At 20°C. the range varied from 300 to 1,900,000 per ml. Since each sample contained 50 ml., the total number of bacteria on the hands in each case was enormous. From the data in Table 1, it is apparent that the bacteria on the hands of food handlers capable of growing at 37°C. particularly is exceedingly large. Fifty per cent of these samples showed a total count in excess of 100,000 per ml.

Coliforms Present. The results of the isolation of coliform organisms from 34 hand-washing samples are given in Table 2. *E. coli* was present alone or in mixed cultures in 13 of the 34 samples examined while *A. aerogenes* was present alone or in mixed cultures in 12 of the 34 samples

TABLE 2

Distribution of Coliform Organisms on Hands of Food Handlers

Type of coliform	Number
E. coli	13
E. freundii	3
A. aerogenes	12
A. cloacae	3
Mixture of *E. coli* and *A. aerogenes*	5
Mixture of *E. coli* and *E. freundii*	1
Mixture of *E. coli* and *A. cloacae*	12
Total samples	34

examined. Coliform organisms could not be isolated in 12 of the 34 samples examined. Most of the negative results were obtained from waitresses and others who were not intimately involved in the preparation or handling of foods.

Hemolytic Streptococci and Staphylococci. The frequency with which hemolytic streptococci and hemolytic staphylococci were isolated from the hands of food handlers is shown in Table 3. The results indicate that hemolytic streptococci and staphylococci were absent in only one sample of 30 that were examined. Twenty-nine of the 30 samples showed hemolytic staphylococci; 19 showed hemolytic streptococci and 19 showed a mixture of hemolytic streptococci and staphylococci. Potentially undesirable bacteria of the coccus type seem to be generally present on the hands of food handlers.

TABLE 3

Distribution of Hemolytic Streptococci and Staphylococci in Washings of Hands of Food Handlers

Hemolytic organism	Number
Streptococcus	19
Staphylococcus	29
Mixture of both	19
None	1
Total samples of washings examined	30

Enteric Bacilli. In an attempt to isolate bacteria of the typhoid-paratyphoid-dysentery group from 34 hand-washing samples by the use of differential media, many suspicious colonies were found. Of these, all except one produced gas when inoculated into lactose broth. This one exception has been tentatively identified as belonging to the Flexner group of dysentery bacilli, since it is impossible to classify enteric disease producing organisms definitely by cultural tests alone and serological identification was not made. The cultural and morphological characteristics of this organism were as follows:

The organism was a Gram-negative, short, plump, non-motile bacillus which produced acid but no gas in glucose and mannitol broths, weak acid but no gas in maltose and xylose broths and no acid or gas in lactose, sucrose, sorbitol, and inositol broths. An acid butt and alkaline slant were obtained on Russel's double sugar agar and on Kligler's iron agar. On the latter medium, H_2S was negative. On bismuth sulfite agar, the colonies were raised and brownish with depressed centers. On MacConkey agar and on Salmonella-Shigella agar the colonies were transparent, uncolored and alkaline. On desoxycholate-citrate agar, the colonies were opaque with even margins.

Aerobic Spore-Forming Bacilli. The total plate counts of aerobic spore-forming bacteria from 34 hand-washing samples are recorded in Table 4. The spore count was made after heating 10-15 ml. of the undiluted sample at 80°C. for 20 minutes.

CONCLUSIONS

The large numbers of bacteria isolated from the hands of food handlers in this investigation and the frequency with which *E. coli*, hemolytic staphylococci and streptococci and aerobic spore formers were isolated in-

TABLE 4

Total Number of Aerobic Spore-Forming Bacilli per ml. of Hand-Washing Sample When Grown on Tryptone Glucose Extract Agar and Incubated at 37° for 48 Hours

Sample No.	Total Count	Sample No.	Total Count	Sample No.	Total Count
1	42	13	11	24	52
2	8	14	10	25	400
3	41	15	5	26	S[1]
4	7	16	12	27	11
5	6	17	8	28	17
6	6	18	27	29	2
7	14	19	16	30	27
8	16	20	23	31	8
9	S[1]	21	S[1]	32	19
10	23	22	11	33	28
11	22	23	28	34	4
12	2	....	....	....	....
				Median:	14
				Range:	4-400

[1] "S" indicates spreader colonies which could not be counted.

dicate the magnitude of the problem of hand hygiene among food handlers and the need for a greatly accentuated campaign of health education for this large and important group of workers.

While it is recognized that many of the organisms isolated in this study were derived from the foods being handled or processed, observation of food handlers has indicated that the hands are frequently soiled with the discharges from the nose and mouth and in other ways. Frequently too, food handlers bring the hands in contact with food when the use of an implement is indicated. This investigation discloses the bacterial condition of the hands of food handlers as found. No attempt was made to discover how they got there. The data justify the conclusion that the hands of food handlers should be kept clean and that they should avoid contact with food whenever possible. Perhaps preliminary instruction of food handlers followed by an examination leading to their licensing will help to bring the problem of effective food sanitation under better control. It is certain, however, that real and lasting progress will not be made until management assumes the responsibility for daily education and supervision.

CHICKEN FLAVOR: THE FRACTIONATION OF THE VOLATILE CONSTITUENTS

ROBERT J. BOUTHILET [a]

Division of Poultry Husbandry, University of California, College of Agriculture, Berkeley, California

[Received for publication, July 29, 1950]

In a preceding paper, Bouthilet (1950), it was shown that the flavor of chicken could be steam distilled from chicken broth. The flavor, when added to a broth of synthetic nonvolatile materials, was indistinguishable from the flavor of the original natural chicken broth. This flavoring material could be further concentrated by fractional distillation.

The present paper deals with the methods of fractionating the chicken flavor.

EXPERIMENTAL

Preliminary Investigations on the Properties of the Flavor Extracts

Since there was no information on the properties of a distillate of cooked meat or poultry, it was necessary to apply various qualitative analytical tests before a concerted attempt to fractionate the components was undertaken.

In all, three distillations were made in the usual process of extraction. The first was made when the chicken broth was steam distilled to yield the volatile flavoring components; this distillate was called the raw distillate. In some cases the raw distillate was put directly into a fractionating column; the distillate from this procedure was termed the flavor concentrate. Finally, when the effect of pH upon the solubility of the flavor became evident, a third distillation was made which was called the acidic distillate.

The Identification of Ammonia as a Volatile Base Occurring in the Raw Distillate and the Flavor Concentrate

It was pointed out in the first paper that the chicken flavoring materials were acidic and only volatile when their water solutions were acidified.

The raw distillate attained in the present work was alkaline to litmus, and when it was concentrated, a definite amine-like odor was noticed. The pH of the original broth was 5, but the raw distillate had a pH of 10; so there must have been a separation of nitrogenous substances during the distillation, possibly in the vapor phase. The alkaline volatile substance must have fixed the flavor, since only a weak odor was evident in the distillate.

In order to identify the volatile amine so evident in the flavor concentrate, a 20-cc. sample was distilled into a solution of sodium hydroxide and benzene-sulfonyl chloride. The usual fractionation technique was carried out to separate primary, secondary, and tertiary amines.

The sulfonamide which separated upon acidification appeared homogeneous and crystalline. After recrystallization until constant melting point, the C, N, H, and S were determined. The melting point and composition indicated that an ammonia derivative had been formed. Known samples were compared and substantiated the identification of the volatile amine as ammonia (Table 1).

Other Qualitative Tests

The flavor in the raw distillate was not extractable under alkaline conditions, even after prolonged periods of extracting.

Before systematic fractionation of the extract was begun, tests were made for several compounds known to be present in meat which might also be present in a steam

[a] Present address: The MacDonald Laboratories, St. Paul, Minnesota.

distillate of meat. Qualitative spot tests were made for the following compounds, according to the methods described by Feigl (1946). Tests for lactic acid, pyruvic acid, esters of carboxylic acids, and aldehydes and ketones were negative, while the tests for carboxylic acids gave positive results. These tests were made to eliminate compounds which might be easily identified and separated as derivatives without further fractionation of the crude distillates.

TABLE 1

Comparison of the Benzene Sulfonamide Derivatives of Ammonia, Methylamine, and the Unknown Compound Obtained from Chicken Broth

	m.p.	C per cent	H per cent	S per cent	N per cent
Unknown sample	154-155°C.	44.8	4.16	20.6	8.25
Ammonia benzene sulfonamide ($C_6H_5SO_2NH_2$)	156°C.	45.2	4.4	20.9	8.6
Methyl amine benzene sulfonamide ($C_6H_5SO_2NH_2CH_3$)	.....[1]	48.8	5.8	18.6	8.2

[1] Not determined.

In any investigation designed to identify micro-constituents from biological materials, the first step is the development of a test for the active substance. In this instance the test was organoleptic (taste). The second step is the development of a procedure for the separation of the active substance in crude form from the main body of inert materials. Steam distillation was suitable for this operation in the chicken flavor study. Finally, the active ingredients must be fractionated into homogeneous components. From this point the classical methods of organic chemistry apply in the establishment of the chemical nature of the substance.

Many attempts to fractionate and concentrate the flavoring materials were made, each one of which added to the knowledge of the properties of the flavor. In the following sections some of the procedures are discussed.

Heavy Metal Precipitation

The raw distillate could be concentrated in a fractionating column. It was distilled twice through a 10-plate, Bruun-type bubble-cap column, giving a 10-fold concentration in each distillation. The 100-fold concentration was accepted as practical, since further distillation in this equipment resulted in flavor destruction by overheating in the boiling pot. There was no provision in the equipment for the injection of liquids at the head of the column. In this fractional distillation there was definite evidence of a clear-cut separation of components. In every case samples from the first 20 per cent of the distillate gave positive nitroprusside tests for sulfhydryl groups, while subsequent collections were negative in this respect. The first fractions were also the portions which had the true chicken flavor while the latter fractions had a more meat-like flavor.

The flavor concentrate, as removed from the receiver after the second run, was placed in an Erlenmeyer flask and a drop of a concentrated solution of mercuric sulfate added. A fine black precipitate formed. The solution was filtered, and again mercuric sulfate added dropwise. At this point a cream-colored precipitate formed. Both the black and the cream precipitates were separately collected and washed with boiled ice-cold water. The black precipitate proved to be mercury sulfide and contained no appreciable amounts of carbon. The cream-colored precipitate contained both sulfur and carbon by qualitative test. The cream-colored precipitate was decomposed with hydrogen sulfide, the mercury sulfide filtered off, and the excess H_2S removed by aspiration with nitrogen gas. A yellow solution was left in the container. This yellow solution had a repulsive odor and gave a positive nitroprusside test, also a positive iodine-azide reaction, indicative of sulfhydryl groups. When this solution was violently shaken or bubbled with oxygen gas, a typical chicken flavor developed which was further enhanced by acidity. The filtrate from the mercuric sulfate precipitation was redistilled in the fractionating column and produced a concentrate of a material which had a typical fatty acid odor. The mercuric sulfate precipitation demonstrated the first definite fractionation of the flavor into a fatty acid fraction and a sulfur-containing portion.

Because of the poor yield in the various stages of fractionation and precipitation in this method it was not adopted for the accumulation of refined flavor. This method has the utility of showing the properties of the flavor.

Solvent Extraction of an Acid Distillate

In order to remove the ammonia which prevented the solvent extraction of the flavor, the original distillate was acidified and again steam distilled. When the receiver was opened a strong odor was evident. This indicated that the flavor was much more volatile than before. Some of the properties of this purified flavor follow: (1) The acidic distillate could be fractionated into two components by simple solvent extraction. The more water-soluble portion had a flavor like that of beef rather than chicken, and so may be described as "meaty" in flavor. The portion easily soluble in solvents had a definite fatty acid odor. (2) A continual desulfuration takes place. Hydrogen sulfide is given off continually from the distillate. (3) The distillate has strong foaming properties.

The acidic distillate was placed in a continuous liquid-liquid extractor and solvent extracted for six to ten hours. The solvent used was isopentane, chosen for the ease with which it could be removed from a water solution and for its low boiling point (28°C.).[1] The extraction was very slow. Complete removal of the flavor was never evident within a 12-hour period.

During the extraction a continual desulfuration was evident. When moistened lead acetate paper was placed above the solvent condenser it blackened quickly, indicating either a breakdown of the compound or a removal of dissolved H_2S from the water by the washing with solvent. The solvent extract was fractionated by adding absolute alcohol, then evaporating the residual isopentane. The alcohol solution was filtered and stored at −20°C. A white substance came out of the solution. A yellow oily material left in the alcohol had an odor of chicken broth. This oil could be further purified by high vacuum distillation in a Hickman still.

The procedure, then, leaves us with three components: a white fatty-acid like substance, a yellow oily fraction, and a water-soluble fraction not extractable with the solvents used. The water-soluble fraction contained considerable flavor. Upon addition of H_2SO_4 to the water-soluble fraction further extraction with the isopentane was evident, as though the acid induced a change in flavor. At no time was the water extract completely odorless. Indications are that the yellow oily material is a breakdown product of the sulfur-containing compound which is not extractable until after the sulfur is removed. Thus the fractionation in this procedure is quite similar to that in the mercuric sulfate precipitation.

Many other procedures, such as aspiration into sodium hydroxide and absolute alcohol, saturation of the solution with pure acetone and freezing out the water, ion exchange adsorption, and continued fractional distillation were tried for purifying the flavoring components. These procedures were abandoned in most cases because the yield of the flavor was too small to be measured. An outline of the chosen procedure is given in the following diagram (Figure 1).

Vacuum Distillation

A description of a third procedure is of interest, because it demonstrates additional properties of the flavor. The vacuum in the flash still was increased to maximum. Steam was allowed to flow freely through the preheater, to distill the solution at a high temperature. A series of traps, cooled by various methods, were aligned before the vacuum pump in the following order: ice and salt, dry ice and acetone, and, lastly, liquid nitrogen. The acidified distillate was introduced and distilled slowly. The high vacuum and high temperature in the heater allowed the condensate to remain warm; so the most volatile portions went into the trapping system. Most of the water was removed in the trap cooled by a mixture of ice and salt; the remainder of the water was condensed in that cooled by dry ice; while the trap cooled by liquid nitrogen removed the remaining volatile materials.

---

[1] Isopentane can be obtained in a very pure grade from Phillips Petroleum Company. The solvent was further purified by shaking with sulfuric acid and distilling.

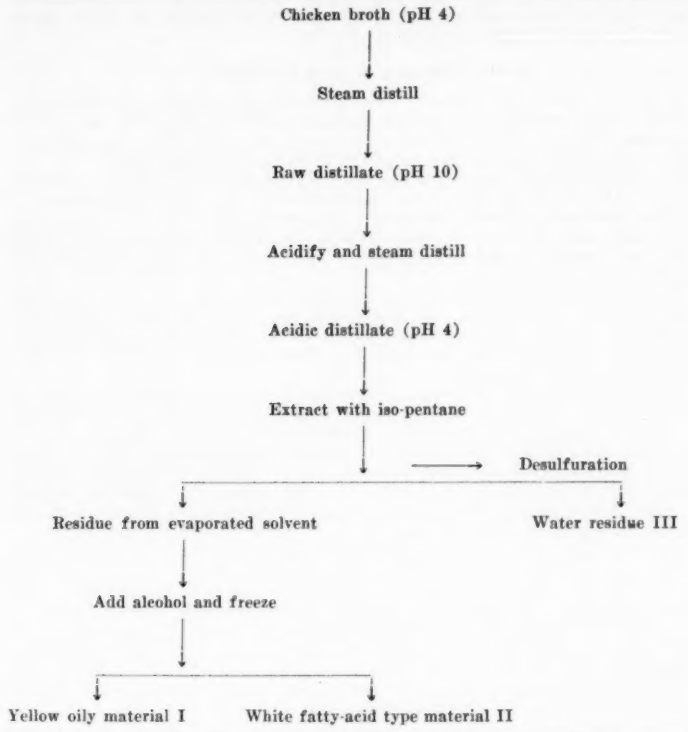

FIG. 1. Procedure for fractionation of chicken-flavor components.

The dry ice trap contained a material with a fatty-acid odor, while the liquid air trap contained a yellow oil which smelled and tasted strongly of chicken. In the liquid air trap some H_2S condensed which was evaporated before tasting the residue.

The volatility of the flavoring substance under the conditions stated, illustrates that its boiling point is very close to that of water. This might account for the difficulty encountered in fractional distillation. The material in the liquid air trap was only partially water-soluble. This change in solubility might have been caused either by the desulfuration or by a breakdown of the flavoring constituents.

DISCUSSION

At this point the following properties of the flavoring compounds are evident: (1) The flavor is composed of at least two portions, namely, a sulfur containing material and a fatty acid-like material. (2) The sulfur compound is highly labile and gives off H_2S upon standing. (3) The sulfur component is produced from some sulfur-containing material of chicken, since no flavor is evident in raw chicken. (4) The flavoring compounds are highly labile and probably cannot be isolated without some destruction.

The desulfuration during distillation of the flavoring materials, and its accompanying changes in properties of the distillate, indicates that it might be very difficult to obtain suitable amounts of the pure flavor in crystalline form. For this reason solvent extraction was chosen to produce a concentrate for identification of the chemical compounds related to the flavor. Identification of these constituents should ultimately lead us to the identity of the original flavoring substance. The other methods of purification discussed are, of course, invaluable to prove that extraction products are not artifacts.

The production of ammonia in the primary distillates is a most interesting and valuable property, for the alkaline conditions fix the flavor in the water solutions, and enable one to collect the flavoring components.

The appearance of two distinct components in the flavor is to be expected. It is of common knowledge and has often been suggested, Crocker (1948), that chicken is composed of two principal flavoring components, a meat flavor, and a flavor peculiar to poultry meat.

In subsequent papers the properties of the isolated compounds will be discussed.

SUMMARY

During the steam distillation of chicken broth there is evident a dissociation of ammonia which condenses and fixes the volatile flavoring components.

Acidification and redistillation produces a strongly flavored distillate. The method chosen for the concentration and fractionation of the components of the distillate was solvent extraction which separated the water solution into three distinct fractions.

Upon standing or extraction with solvent, desulfuration (hydrogen sulfide production) of the distillate continues as long as the true flavor is evident. That sulfur is an integral part of the flavor is demonstrated by preparation of the true odor by heavy metal precipitation.

The decomposition of the flavor during solvent extraction indicates that a study of the breakdown products will be essential to identification of the contributing component.

REFERENCES

BOUTHILET, R. J., 1950. Chicken flavor: Separation and concentration of its volatile components from broth. Food Research **15**, 322.

CROCKER, E. C., 1948. The flavor of meat. Food Research **13**, 179.

FEIGL, F., 1946. Qualitative analysis by spot tests. Elsevier Publishers, Inc., New York.

CONSTITUTION OF THE JUICE LIPIDS OF THE FLORIDA VALENCIA ORANGE (*CITRUS SINENSIS* L.)[1]

LYLE JAMES SWIFT AND M. K. VELDHUIS
U. S. Citrus Products Station,[2]
Winter Haven, Florida

[Received for publication, August 4, 1950]

It has long been suspected that part of the deterioration in the flavor of canned orange juice on storage is due to changes in the lipid constituents. Nolte and von Loesecke (1940) established that 16 months of storage of canned juice caused oxidative changes in the lipid fraction. Matlack (1940) made a qualitative analytical study of California Valencia orange juice lipids, but did not attempt to study changes that occurred on storage. Swift (1946) devised an analytical method for the determination of lipid materials in orange juice. Curl (1946) concluded from storage studies on filtered and unfiltered orange juice that the lipid substances played an important role in flavor deterioration. The object of the present work was to isolate a sufficient quantity of the lipid so that a somewhat more detailed study of the composition might be made and a better understanding of the changes become possible.

EXPERIMENTAL

In the 1947 Valencia orange season, 116 boxes of oranges, mostly packing-house rejects, were hand-reamed to extract the juice which was then passed through a commercial finisher, deaerated, pasteurized at about 95°C., and sealed in 46-ounce plain tin cans. In all, 1644 cans of the 46-ounce size and 21 cans of the 18-ounce size were obtained. Each day's pack was divided into two equal lots, one of which was stored in a laboratory loft at temperatures ranging up to 40°C., while the other was stored in the cold room at 0° to 5°C. The present work is concerned with the examination of the lipids of the canned juice that had been stored in the refrigerator. After further study it is planned to report the results of a similar examination of the lipids of the aged juice.

As soon as was convenient, the extraction of the freshly canned juice (i.e., that stored in the cold room) was begun. Previous work by Swift (1946) had shown that filtering and extraction of the filter cake was the most feasible way to do this. Since the equivalent of 830 cans of the 46-oz. size were to be processed, it was necessary to carry out the extraction batchwise, nearly 70 runs being required. One dozen 46-ounce cans were processed at a time, the contents being bulked, treated with about 70 gm. of Hyflo Supercel filter-aid,[3] and filtered on two 10-inch Buchner funnels that had been precoated with the same material. The filter cakes were drawn as dry as possible by vacuum and then were cut into 1-cm. squares with a sharp spatula while still in the funnel. The pieces of filter cake so obtained were then charged into a stainless steel extractor of the Soxhlet type and extracted with acetone for about 20 hours or until the acetone from the extraction chamber was colorless. Previous work had shown that practically all the lipid substances are extracted by acetone under these conditions so a subsequent extraction of the filter aid with petroleum ether was not made. The acetone was distilled from the extract and the residue was taken up in petroleum ether, which

[1] Agricultural Chemical Research Division Contribution No. 263.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[3] The mention of trade products does not imply their endorsement by the Department of Agriculture over similar products not mentioned.

was then combined with the petroleum ether extracts from the other batches. Upon concentration of this total extract, a solid material separated which, after recrystallization from a large volume of ethanol and drying, weighed about 26 gm. and gave the reactions of a sterol glycoside. This conclusion has since been confirmed and this substance has been reserved for further study. When the volume of the remaining solution had been reduced to 4.5 liters, an aliquot was measured into a tared flask and the weight of lipid obtained by evaporation, drying, and weighing. From the data so obtained it was calculated that about 1000 gm. of lipid material had been obtained. This represents a yield of about 0.09 per cent on the whole juice basis.

The bulk of the petroleum ether was distilled from a portion of the extract and the residue was heated for 2 hours in a vacuum at 95°-100°C. to remove the last traces of the solvent. The resulting lipid material was a dark-red viscous liquid and it is upon this that the analyses in this paper are based. In Table 1 are listed the results of the determinations, without any attempt at interpretation.

TABLE 1

Analysis of Lipid Fraction from Florida Valencia Oranges

Iodine no. of esters of fatty acids	107.3
Saponification equivalent of whole lipid	336.0
Unsaponifiable	14.81 per cent
Volatile in steam	0.17 per cent
Fatty acids, total	59.00 per cent
Fatty acids, free	21.00 per cent
Glycerol	3.43 per cent
Phosphorus	1.40 per cent
Nitrogen	0.61 per cent
Choline	2.36 per cent
Isoamyl alcohol soluble (resin acids?), total	12.41 per cent
Isoamyl alcohol soluble (resin acids?), free	4.90 per cent
Additional sterol glycoside	1.48 per cent

The unsaponifiable fraction was determined by the Kerr-Sorber procedure and fatty acids were determined on the residue in the usual manner. A detailed study of their composition is being made and the results will be published separately. Glycerol was determined on the aqueous solution after extraction of the acids by a procedure adapted from one in the A.O.A.C. Official and Tentative Methods, 6th edition, page 551. Phosphorus was determined by standard methods after ashing the lipid with magnesium nitrate. Nitrogen was determined by the Kjeldahl method on an acid hydrolysate after the unsaponifiable matter and acids had been removed. Choline was determined on the acid hydrolysate of the lipid, using the method of Thornton and Broome (1942). The isoamyl alcohol-soluble material was obtained by extraction with this solvent of the acidified aqueous saponification residue after the removal of unsaponifiable and fatty acids by petroleum ether. This is the material referred to by Nolte and von Loesecke (1940) as "acid resins." The determination of the steam-volatile material was made by introducing a sample of the lipid into a liter flask, adding a large volume of water, and distilling in a volatile oil apparatus. The free fatty acids were obtained by titrating a sample of the lipid in neutralized ethanol, diluting with water and petroleum ether, shaking, and allowing the layers to separate in a separatory funnel. The aqueous solution of the acid salts was withdrawn and the petroleum ether solution of the residual lipid was washed a couple more times with water, the washings being added to the aqueous solution which was then made acid and extracted with petroleum ether. The fatty acids extracted by this solvent were determined in the usual way by evaporating, drying under carbon dioxide, and weighing in a tared flask. The acids were highly colored and obviously impure. The aqueous solution remaining was then extracted with isoamyl alcohol. This solution was handled in the same manner as the petroleum ether extract, except that it was dried in a vacuum oven until the odor of isoamyl alcohol was not perceptible. The weight expressed as a percentage was entered as "free isoamyl alcohol soluble." The item entitled "additional sterol glycoside" was not obtained

from an analytical sample, but accumulated at the interfaces when 600 gm. of the lipid was extracted for fatty acids after removal of the unsaponifiable matter. After recrystallization from pyridine and alcohol and drying, the weight was 8.9 gm., or 1.48 per cent.

DISCUSSION

Obviously the amounts of some of the constituents as determined require some interpretation if one is to arrive at the probable state in which they existed in the unsaponified lipid. For example glycerol could not occur free because it would be water-soluble and be lost in the juice. Besides, there are more than enough acids to combine with the glycerol so it is likely that it exists as the glyceryl radical and to the extent of 1.53 per cent. Probably the best point from which to attack the problem is from the phosphorus and nitrogen content. It will be observed that these occur in amounts of 1.40 and 0.61 per cent, respectively, which are in the atomic ratio of 1:1, commonly found in phosphatides such as lecithin and cephalin. This ratio, plus the fact that choline was found and determined, makes it extremely likely that ethanolamine is present and accounts for the balance of the nitrogen. An attempt was made to demonstrate its presence using the method of Shupe (1941). The aqueous portion of the acid hydrolysate was concentrated somewhat and treated with sodium nitroprusside solution and a positive test for a primary amine was obtained. An attempt was made to prepare the p-bromo-benzene sulfonyl chloride derivative which for ethanolamine should melt at 94°C. A small amount of crystalline material melting at 86°C. was obtained. By repeated recrystallizations the melting point was raised to 90°C., but the amount of material was too small to carry the purification further. Thus, while the presence of ethanolamine is strongly indicated, it is not proved, but will be included as the most likely compound to account for the balance of the nitrogen. The phosphorus was almost certainly present as the monoacid phosphate radical, which is a part of the phosphatide molecule, and the equivalent percentage of this is 4.34. The choline, calculated as the cholinyl radical, is 2.03 per cent instead of 2.36 per cent. The nitrogen, not accounted for as choline, gives 1.09 per cent when calculated as the ethanolaminyl radical. The average molecular weight of the fatty acids, 273 (from further work to be published later), would be 272 as the acid radical and this would alter the combined acid percentage from 38.0 to 37.9. A similar adjustment should be made on the combined portion of the acid resins, but it is small and uncertain and will be omitted. The total is given in Table 2. Some of the sterols of the unsaponifiable may be combined with fatty acids.

The 3.24 per cent of the lipid fraction unaccounted for may represent experimental error. There is also a possibility that inositol, which has been found in citrus fruit by Nelson and Keenan (1933) and by Cheldelin and Williams (1942) is a constituent of the lipid fraction as part of the phosphatide portion. It has been found as a phosphatide constituent in other sources.

By taking the average molecular weight of the fatty acids as 272, it is possible to calculate the amounts of lecithin and cephalin from the amounts of choline and ethanolamine, respectively. Thus, 2.03 per cent

TABLE 2

Composition of the Lipid by Radicals and Classes of Compounds

Fatty acids, combined			37.90 per cent
Fatty acids, free			21.00 per cent
Unsaponifiable matter			14.81 per cent
Isoamyl alcohol-soluble (resin acids) total			12.41 per cent
Additional sterol glycoside			1.48 per cent
Volatile in steam			0.17 per cent
Glyceryl radical $CH_2—CH—CH_2$			1.53 per cent
Monoacid phosphate radical, HPO_4			4.34 per cent
Nitrogen, total	0.613 per cent		
Choline N	0.273 per cent	Cholinyl radical	2.03 per cent
Ethanolamine N	0.340 per cent	Ethanolaminyl radical	1.09 per cent
		Total	96.76 per cent

of the cholinyl radical implies 15.3 per cent lecithin and 1.09 per cent of the ethanolamine radical implies 18.0 per cent cephalin. These account for a little more glycerol than was actually found. This still leaves 13.6 per cent of the combined acids that may be combined with sterols, a possibility previously pointed out by Matlack (2). There is no direct evidence for this, however. When the above relationships are taken into account, the composition of the lipid may be expressed as shown in Table 3.

TABLE 3

Probable Composition of Lipid as Interpreted from Data

Unsaponifiable matter	14.81 per cent
Isoamyl alcohol-soluble (resin acids) total	12.41 per cent
Fatty acids, free	21.00 per cent
Fatty acids, combined (perhaps with sterols)	13.60 per cent
Sterol glycoside	1.48 per cent
Volatile in steam	.17 per cent
Lecithin	15.3 per cent
Cephalin	18.0 per cent
	96.77 per cent

SUMMARY

1. The lipid material of Florida Valencia orange juice has been isolated and a proximate analysis has been made. It appears that the particular sample isolated contained about one-third phosphatides in spite of the fact that the extraction of them was made with acetone.

2. The percentage of unsaponifiable matter is considerably lower than has been found on earlier samples indicating that the lipid constitution is subject to considerable variation.

REFERENCES

CHEILDELIN, V. H., AND WILLIAMS, R. J., 1942. The B Vitamin Content of Foods. Univ. of Texas Publication 4237, pp. 105-124.

CURL, A. L., 1946. Off-flavor development in processed tangerine juice. Fruit Products J., 25, 356-357.

MATLACK, M. B., 1940. The fatty constituents of California valencia orange pulp. J. Org. Chem., **5**, 504-507.

NELSON, E. K., AND KEENAN, G. L., 1933. i-Inositol in citrus fruits. Science, **77**, 561.

NOLTE, A. J., AND VON LOESECKE, H. W., 1940. Chemical and physical characteristics of the petroleum ether soluble material of fresh and canned Florida Valencia orange juice. Food Research, **5**, 457-467.

SHUPE, J. S., 1941. Detection and determination of mono- and di-ethanolamines. J. Assoc. Off. Agric. Chem., **24**, 754-757.

SWIFT, L. J., 1946. The determination of crude lipid in citrus juices. J. Assoc. Off. Agric. Chem., **29**, 389-395.

THORNTON, M. H., AND BROOME, F. K., 1942. Determination of choline. A photometric modification of Beattie's method. Ind. Eng. Chem., Anal. Ed., **14**, 39-41.

QUALITY OF ARTIFICIALLY RIPENED TOMATOES FOR CANNING PURPOSES

JOHN SKOK[1]
Department of Horticulture, University of Illinois, Urbana, Illinois

[Received for publication, August 8, 1950]

Tomatoes produced for the canner are harvested at the red-ripe stage as maximum color is required. This conventional procedure, however, does not insure highly colored tomatoes during all periods of the growing season and is also partially responsible for the high losses sustained in the field. The red tomato pigment (lycopene) develops most rapidly in the temperature range of 21-24°C.(70-75°F.), Duggar (1913), Wright, Pentzer, Whiteman, and Rose (1931), and Vogele (1937), and its rate of formation decreases rapidly as the temperature drops below or rises above this rather narrow range. The temperature of a tomato fruit, when adequately shaded by foliage, will be approximately that of the air temperature. The temperature of a fruit exposed to the sun, however, will be considerably higher than air temperature. A number of measurements have indicated that fruits exposed to the sun will often reach temperatures as high as 38-49°C. (100-120°F.) during bright days. Fruit temperatures of 32°C.(90°F.) are quite common during the day time. Since fruit temperatures are predominantly in the nineties during the latter part of July and August, and often in the sixties or below during the latter part of September, conditions for optimum coloration are unfavorable during a large portion of the tomato season. This means that the fruits must remain on the plant longer for lycopene accumulation than they would if optimum temperatures prevailed. Over-ripe and soft fruits often result, and a high proportion is lost. Losses sustained in the field vary with temperature and rainfall, and are usually high. Often only half the crop is harvestable. It is obvious that the longer the fruit must remain in the field the greater these losses will be; and conversely, the earlier the fruits are harvested the greater will be the recovery.

Gaylord and MacGillivray (1934) observed over 25,000 tagged fruits to determine their ripening sequence in the field. They found that total fruits reaching U. S. No. 1 cannery grade in Indiana may be as low as 28.8 per cent during adverse seasons and the average for a five-year period was only 68.3 per cent. These percentages represent the total fruits reaching the U. S. No. 1 condition, i.e., the highest grade attainable. Under commercial harvesting conditions, however, many of these fruits are not harvested at this grade. The percentage actually taken from the field would be considerably less, due to field losses caused by cracking, rotting, and sun scalding.

Immature fruits may be ripened off the vine and, if optimum ripening temperatures are provided, high lycopene will develop. Artificial ripening

[1] Present address: Biology Division, Argonne National Laboratory, Chicago, Illinois.

of tomatoes is a common practice with Southern- and Mexican-grown "green-wrap" fruits. The procedure and problems have been described by Sando (1920), Diehl (1924), Rosa (1925, 1926), and Wright *et al.* (1931, 1940). Artificial ripening of immature tomatoes for canning purposes has not been given general consideration. The purpose of the experiments here reported is to determine the relative quality of vine-ripened and artificially ripened tomatoes for canning uses.

DEFINITIONS

To avoid repetition in description of maturity stages, certain terms used will be defined. During ripening of the tomato several changes in composition and texture occur simultaneously with pigment changes, but the following terms pertaining to maturity stages are based on the color of the fruit. A green fruit attains maximum size; then chlorophyll disappears and carotinoids develop. The term "mature-green" refers to the stage when the fruit is green, but sufficient decomposition of chlorophyll has taken place at the blossom end to produce a white or very light green area. No trace of red color has developed. This is succeeded by the "turning stage" when the fruit is still mostly green, but the blossom end area has a slight blush of red color. Fruits at the "pink stage" have passed the "turning stage" but have not reached the U. S. No. 2 stage. They are still green but have a definite pink color. The color of U. S. No. 2 and U. S. No. 1 stages are those defined by the Bureau of Agricultural Economics in their U. S. standards for canning tomatoes. A U. S. No. 2 fruit, with reference to color, must be "fairly well colored" which means that the tomato shows at least two-thirds good red color. A U. S. No. 1 fruit must be "well colored" which means that the tomato shows at least 90 per cent good red color. The U. S. No. 1 stage is also often referred to as the red-ripe stage.

To avoid confusion concerning grade of canning tomatoes, an explanation may be desirable. Frequently, tomatoes delivered to the cannery may grade 90 per cent or more U. S. No. 1 fruits, and relatively few U. S. No. 2 and cull fruits. This breakdown of grades, however, is based on the load delivered to the cannery and has no direct relationship to the relative proportions of these grades in the field. Obviously, the lower grade and cull fruits are not harvested but left in the field.

PROCEDURE AND METHODS

The effect of various harvesting procedures on yield was tested during the 1944 season, and various preliminary ripening studies were also conducted in Urbana during the 1944 and 1945 seasons. During the 1946 and 1947 seasons, larger scale ripening studies were conducted in the northern part of the state in cooperation with a canner. Sufficient tomatoes of the three canning varieties, Rutgers, Garden State, and Stokesdale, were planted in 1947 to operate a one-line tomato-juice plant. About 20,000 cases of commercial tomato juice were packed from vine-ripened and artificially ripened tomatoes. The tomatoes ripened artificially were harvested at both turning and pink stages and ripened in lug boxes stacked in a ripening room large enough to hold 45 tons of tomatoes at one time. The temperature of the ripening room was held at 22°C. (72°F.) and the air in the room was continuously renewed by a circulating fan and duct system to provide adequate ventilation.

Duggar (1913), Vogele (1937), and Clendenning (1941, 1942) have shown that oxygen is required for ripening and pigment formation. Sando (1920) has pointed out that absence of proper ventilation during ripening will result in inferior flavor in tomatoes.

Samples of juice of each lot canned were analyzed in the Vegetable Crops laboratory of this department for total solids, total sugars, pH, ascorbic acid, total pigments (primarily lycopene), and carotene. All determinations were made on duplicate samples.

Total solids were determined on twenty-gram samples in a ventilated Brabender oven adjusted at 80°C.(176°F.). Recorded total solids are corrected to exclude the salt added to the juice.

Total sugars were determined by the picric acid method as described by Willaman and Davison (1924), but the determinations were made with a filter photometer previously standardized with dextrose.

A glass electrode was used to determine pH.

A 0.5 per cent oxalic acid solution was used to extract ascorbic acid as recommended by Ponting (1943). Mixtures containing one ml. of acid for each gram of juice were blended. Filtered aliquots were titrated with standardized sodium 2,6-dichlorobenzenoneindophenol.

Total pigments were extracted with hexane and determined on a filter photometer previously standardized with a prepared sample of lycopene.

Carotene was separated from an aliquot of the total pigment extract by adsorption on a Tswett column consisting of a mixture of magnesium oxide and a diatomaceous filter aid (Hyflo Super-Cel, Johns Manville) and eluted with a few drops of absolute ethanol. The eluent removes the carotene first which is made up to volume and similarly determined with the photometer standardized for carotene.

The differences between the analyses of the juices made from vine-ripened and room-ripened fruits were tested for significance by the *t* test and handled according to the procedures for comparing two unpaired groups with unequal numbers.

RESULTS

Harvest procedure and yield. As already stated, the yield of tomatoes is determined, among other production factors, by the number of usable fruits which are recovered. Recovery of fruits decreases in relation to the time during which they remain on the vine. Any practice which decreases the time a fruit remains on the vine would also increase the yield of usable tomatoes.

During the 1944 season, tests were conducted at the Cook County Station, Des Plaines, Illinois and at Urbana to observe the effects of harvesting immature fruits and frequency of harvesting on yield. Since many of the unusable fruits remaining in the field are cracked and partially or entirely decayed, the effect of removal of culls was also tested. The results (Tables 1 and 2) indicate that both frequency of harvesting and stage

TABLE 1

Effect of Harvesting Procedure on Yield of Tomatoes[1]

Harvested at turning stage, culls removed	Harvested at U. S. No. 2 stage, culls removed	Harvested at U. S. No. 1 stage, culls removed	Harvested at U. S. No. 1 stage, culls not removed
tons per acre	*tons per acre*	*tons per acre*	*tons per acre*
13.63	12.78	9.82	7.52

[1] Cook County plots, Early Baltimore variety. Yields based on four replications. Tomatoes harvested twice per week or as needed.

TABLE 2

Effect of Harvest Frequency and Stage of Maturity at Time of Harvest on Yield of Tomatoes[1]

Variety	Harvested six times per week at U. S. No. 2 stage, culls removed	Harvested six times per week at U. S. No. 1 stage, culls removed	Harvested two times per week at U. S. No. 1 stage, culls not removed	Harvested once per week at U. S. No. 1 stage, culls not removed
	tons per acre	*tons per acre*	*tons per acre*	*tons per acre*
Early Baltimore	30.60	23.91	14.14	12.19
Illinois T19	21.22	14.47	6.23	3.31

[1] Urbana plots.

of maturity at time of harvesting affect yield. The increases in yield due to harvesting at immature stages as compared with harvesting at the red-ripe stage range from approximately 28 to 40 per cent. The yields are still further increased when the picking frequency is increased and when culls are removed from the field. Recently Sayre and Patterson (1950) have demonstrated similar consistent increases in yield by harvesting at immature stages.

Artificial ripening of turning and pink fruits. Fruits ripened artificially for the "green-wrap" industry are harvested at the "mature-green" stage. Such fruits require a long time to develop red color. Coloration proceeds at a rather non-uniform rate, because mature-green fruits actually represent a rather wide range of maturity. Preliminary experiments also indicated that mature-green fruits, ripened artificially, will not attain a color suitable for canning purposes.

Fruits selected at the "turning stage," or at any succeeding stage, may be ripened satisfactorily. Such fruits will develop a high color. Turning fruits ripen very uniformly and with little variation in red color, because the turning stage represents a definite and easily recognized stage of maturity in that the red color is just appearing.

A turning fruit requires about 12 days to attain maximum or near maximum color under optimum ripening conditions [about 22°C.(72°F.) and adequate ventilation]. A pink fruit requires several days less, depending upon its actual maturity stage. Good color, however, can be obtained in considerably shorter periods and under commercial conditions it may be advantageous to reduce the time in the ripening room and accept a slight loss in pigment formation.

Fruits ripened for commercial uses should be sorted into comparable color stages to assure uniform ripening and to prevent the development of over-ripe soft fruits, which may start to decay before other fruits have reached a satisfactory stage.

Fruits ripened off the vine lose weight, primarily due to moisture loss. During the 1946 season the magnitude of such moisture loss was determined for 43 different lots. These lots were made up of turning and pink fruits of the Rutgers and Illinois T19 varieties, which were placed in the ripening room for various periods. The lots, consisting of five to 20 lug boxes each, were weighed before and after ripening. A lug box contained about 35 to 40 pounds of fruit. The average loss from the 43 different lots was 5.65 per cent for an average time of 145 hours, or slightly less than 1 per cent per day (0.93 per cent per day or about 0.039 per cent per hour). The average moisture loss of turning tomatoes was 0.04 per cent per hour and that for pink tomatoes was 0.037 per cent per hour. A loss this small was not sufficient to cause noticeable shriveling during the ripening period. MacGillivray (1926) has shown that weight losses during storage of tomatoes, in the case of fully ripe fruits, are proportional to the length of time in storage as well as the temperatures. He also detected some losses in organic constituents.

Analyses of canned juice. Two canned samples of each lot canned during the 1947 season were bulked and analyzed in duplicate. A total of 106

separate lots were analyzed, of which 58 were juice made from field-ripened tomatoes and 48 were juice made from room-ripened tomatoes. A statistical summary of the results is given in Table 3.

TABLE 3

Comparative Composition of Tomato Juice Made from Vine-Ripened and Room-Ripened Tomatoes

	Ascorbic acid (mg. per 100 gm.)		Total solids, pct.		Total sugars, pct.		Total pigments as lycopene (mg. per 100 gm.)		Carotene (mg. per 100 gm.)		pH	
	Vine ripe	Room ripe	Vine ripe	Room ripe	Vine ripe	Room ripe	Vine ripe	Room ripe	Vine ripe	Room ripe	Vine ripe	Room ripe
Mean values	21.27	17.97	5.93	5.94	3.43	3.27	6.02	5.63	.285	.311	4.2	4.2
Standard error of mean	0.20	0.29	0.04	0.05	0.06	0.06	0.16	0.17	.005	.002	.012	.015
Coefficient of variation, pct.	7.34	11.11	5.39	6.12	13.18	13.41	19.92	20.93	12.62	3.59	2.15	2.45
Significance at one per cent level	Yes		No		No		No		Yes		No	

The ascorbic acid content of canned juice made from field-ripened tomatoes was 21.27 ± 0.20 mg. per 100 grams as compared to 17.97 ± 0.29 mg. per 100 grams for juice made from room-ripened tomatoes, a highly significant increase of almost 19 per cent as indicated in Table 3. Such a difference would be expected since room-ripened tomatoes receive no sunlight during the ripening period. Hamner and Maynard (1942), McCollum (1944), and others have shown that exposure to sunlight will increase ascorbic acid in tomatoes.

The total pigment content of a tomato is an index of red color since most of the total pigment consists of lycopene. Carotene, a yellow pigment, however, will also influence the color. The total pigment content of juices made from vine-ripened and room-ripened tomatoes do not differ significantly (Table 3).

The carotene content of juice made from room-ripened tomatoes was 0.311 ± .002 mg. per 100 grams as compared to 0.285 ± .005 mg. per 100 grams for juice made from vine-ripened fruits, a highly significant increase of over nine per cent. This finding is somewhat contrary to what might be expected as sunlight exposure has also been found to increase carotene in tomatoes. Ellis and Hamner (1943) have reported a reduction of carotene in tomatoes ripened artificially as compared to that in vine-ripened fruits. The larger amount of carotene in juice made from room-ripened fruits in the present experiments may be due in part to the fact that room-ripened tomatoes were of uniform ripeness and were processed before becoming over ripe. Over-ripe fruits are low in carotene and a certain portion of field-ripened fruits are usually over ripe. This is emphasized by the greater uniformity in carotene content of juice made from

room-ripened tomatoes as compared to that made from vine-ripened tomatoes. The coefficients of variation (Table 3) for the room-ripened group is 3.59 per cent as compared to 12.62 per cent for the vine-ripened group.

The differences between total solids, total sugars, and pH values of juices made from field-ripened and room-ripened tomatoes were not significant. The pH values were very uniform (coefficients of variation are 2.15 per cent and 2.45 per cent, respectively) and the values for the two groups are substantially identical (4.2 ± 0.012 and 4.2 ± 0.015).

It would be expected that total sugars (and total solids, as affected by total sugars) would be lower in the room-ripened series because of sugar lost during respiration of fruits while in the ripening room. This is, no doubt, offset by the moisture loss of this group while in the ripening room. There is no direct way to detect absolute amounts of sugar lost. As shown in Table 3, the differences between total sugars of the two series (3.43 ± 0.06 and 3.27 ± 0.06) are greater than the differences between total solids (5.93 ± 0.04 and 5.94 ± 0.05) but neither difference is significant.

These results indicate that immature tomatoes can be ripened for canning purposes. With the exception of ascorbic acid, the quality of such tomatoes is equal to or superior to vine-ripened fruits. Juice samples of canned lots were tasted and no detectable differences were observed. The graders also graded the juices made from artificially ripened fruits as high or higher than those made from field-ripened fruits. The field-ripened tomatoes used for making juice, of course, were selected for the usual canning requirements and a considerable proportion of the field-ripened fruits could not be used. If this selection is not made the resulting juice would be of an inferior grade and the extent of the poorer grade will vary with the season. Sando (1920) reported that artificially ripened tomatoes were equal in quality to vine-ripened fruits, provided adequate ripening conditions are maintained. Since the controlled environment of a ripening room is uniform and adjusted to meet optimum ripening conditions, it is possible to produce tomatoes and tomato products that are much more uniform than those produced from tomatoes grown under variable ripening conditions in the field. Saywell and Cruess (1932) and Hamner and Maynard (1942) show that quality constituents vary considerably in tomatoes and tomato products, depending on the conditions under which the crop is grown.

SUMMARY

Yields of usable tomato fruits are increased by harvesting the fruits at various immature stages as compared to harvesting at a red-ripe stage. This increase in yield is brought about by a reduction of the losses normally sustained when fruits are permitted to ripen fully on the vine. Fruits harvested at the turning stage, or at any later stage, may be ripened to a fully colored fruit under adequate ripening conditions. Fruits harvested in the green condition, with no trace of red color, do not ripen uniformly, nor do they develop maximum color. The moisture lost from the fruits during artificial ripening is equal to about one per cent per day. This amount was not sufficient to cause noticeable shriveling in any of these experiments.

Canned tomato juice was prepared under commercial conditions from 106 different lots of tomatoes. Of these, 58 lots were field-ripened fruits and 48 were fruits room-ripened from both the turning and the pink stages. Canned samples of each lot were analyzed for ascorbic acid, total solids, total sugars, total pigments, carotene, and pH. The ascorbic acid content of juice made from vine-ripened fruits was about 19 per cent higher than that of juice made from room-ripened fruits. The carotene content of juice made from room-ripened tomatoes was about 9 per cent higher than that of juice made from vine-ripened fruits. There were no significant differences in total pigments, total solids, total sugars, and pH values of the juices made from field-ripened and room-ripened tomatoes. The color of the juices of the two lots was comparable.

Acknowledgments

The author is thankful to Mr. C. Y. Arnold who obtained the yield data at the Cook County plots in 1944; to Libby McNeill & Libby for support of certain cooperative work during 1944 and 1945; and to the California Packing Corporation for support of the work during 1946 and 1947, and for their provision of growing, ripening, and canning facilities and services.

REFERENCES

CLENDENNING, K. A., 1941. Studies of the tomato in relation to its storage. II. The effect of altered internal atmosphere upon the respiratory and ripening behavior of tomato fruits stored at 12.5°C. Can. J. Research, **19**, 500-518.

———, 1942. The respiratory and ripening behavior of the tomato fruit on the plant. Can. J. Research, **20**, 197-203.

DIEHL, H. C., 1924. The chilling of tomatoes. U. S. Department of Agriculture, Circular **315**.

DUGGAR, B. M., 1913. Lycopersicin, the red pigment of the tomato, and the effect of conditions upon its development. Wash. Univ. Studies, **1**, 22-45.

ELLIS, G. H., AND HAMNER, K. C., 1943. The carotene content of tomatoes as influenced by various factors. J. Nutrition, **25**, 539-553.

GAYLORD, F. C., AND MACGILLIVRAY, J. H., 1934. Tomato quality studies. Field and harvest factors affecting grade. Purdue Agr. Expt. Station Bull. **394**.

HAMNER, K. C., AND MAYNARD, L. A., 1942. Factors influencing the nutritive value of the tomato. A review of the literature. U. S. Department of Agriculture, Misc. Pub. No. **502**.

MACGILLIVRAY, J. H., 1926. Studies of tomato quality and the effect of temperature on storage losses. Proc. Am. Soc. Hort. Sci., **23**, 208-214.

MCCOLLUM, J. P., 1944. Some factors affecting ascorbic acid content of tomatoes. Proc. Am. Soc. Hort. Sci., **45**, 382-386.

PONTING, J. D., 1943. Extraction of ascorbic acid from plant materials. Relative suitability of various acids. Ind. Eng. Chem., Anal. Ed., **15**, 389-391.

ROSA, J. T., 1925. Ripening of tomatoes. Proc. Am. Soc. Hort. Sci., **22**, 315-322.

———, 1926. Ripening and storage of tomatoes. Proc. Am. Soc. Hort. Sci., **23**, 233-242.

SANDO, C. E., 1920. The process of ripening in the tomato, considered especially from the commercial standpoint. U. S. Department of Agriculture, Bull. **859**.

SAYRE, C. B., AND PATTERSON, M. E., 1950. Comparative yields of "green-wrap" and red ripe tomatoes. Farm Research, **16**, 7.

SAYWELL, L. G., AND CRUESS, W. V., 1932. The composition of canning tomatoes. Cal. Agr. Expt. Station Bull. **545**.

VOGELE, A. C., 1937. Effect of environmental factors upon the color of the tomato and the watermelon. Plant Physiol., **12**, 929-955.

WILLAMAN, J. J., AND DAVISON, F. R., 1924. Some modifications of the picric acid method for sugars. J. Agr. Research, **28**, 479-488.

WRIGHT, R. C., AND GORMAN, E. A., JR., 1940. The ripening and repacking of mature green tomatoes. U. S. Department of Agriculture, Circular **566**.

———, PENTZER, W. T., WHITEMAN, T. M., AND ROSE, D. H., 1931. Effect of various temperatures on the storage and ripening of tomatoes. U. S. Department of Agriculture, Tech. Bull. 268.

THE EFFECT OF CERTAIN PREFREEZING TREATMENTS ON THE QUALITY OF EIGHT VARIETIES OF CULTIVATED HIGHBUSH BLUEBERRIES

MARY L. GREENWOOD AND MARTHA POTGIETER
School of Home Economics, Storrs Agricultural Experiment Station, University of Connecticut, Storrs, Connecticut

AND

C. I. BLISS
Connecticut Agricultural Experiment Station, New Haven, Connecticut

[Received for publication, August 18, 1950]

Cultivated blueberries, though relatively new, are becoming more popular in the family garden. There is still no general agreement as to which varieties are the most satisfactory for home use. Moreover, when they are to be frozen, the best form of sweetening and whether or not scalding is advisable have not yet been established. The present paper concerns the relative palatability of eight varieties of cultivated highbush blueberries and the effects of freezing by commonly accepted home procedures without sweetening, with dry sucrose, and with sucrose sirup, and, in each case, with and without scalding.

EXPERIMENTAL PROCEDURE

The tests were begun in the summer of 1947, all the berries being picked near the middle of the harvest season. The varieties chosen were the highbush hybrids Atlantic, Cabot, Concord, Jersey, Pemberton, Pioneer, and Wareham, as well as Rubel, a varietal selection from the wild. They included both early and late types and those of commercial importance in New England.

Immediately after picking, the unripe, overripe, and split berries were sorted out, weighed, and discarded. In certain instances the weight of 100 berries was determined. After sorting, the berries were washed and drained. Weighed portions from each day's picking were subjected to six different treatments preparatory to freezing, as indicated below:

Treatment	Unsweetened berries, gm.	Dry sucrose		Sucrose sirup		
		Berries, gm.	Sucrose, gm.	Berries, gm.	Sirup, gm.	Concentration of sirup, per cent
Not scalded	350	350	70	200	155	35.5
Scalded	350	350	70	200	155	35.5

The dry sugar was sprinkled over the berries and the mixture stirred frequently until the sugar was dissolved. The 35.5 per cent sirup was used in all samples, except in the first four tests in which two concentrations of 33.3 and 37.5 per cent were used. For scalding, the berries were placed in a colander and scalded in steam over boiling water for 1 min. in a covered container, not under pressure. This method was chosen as one which could easily be duplicated in the home.

The ratio of sugar to berries in the dry pack was 1:5 by weight, a commonly recommended level for blueberries. The 35.5 per cent concentration of sugar in the sirup corresponded in sweetening to that of the dry pack. Sirups slightly more dilute and more concentrated were tried in the earlier tests, but in the absence of a consistent

preference, a concentration of 35.5 per cent was adopted for all later experiments. This sirup is lighter than either the 40 per cent which is often recommended for freezing blueberries or the 50 per cent sirup preferred by Moon, Smart, and Caldwell (1936).

The berries in sirup were put into pint jars and the others into cellophane bags and all frozen and stored at −18°C.(0°F.). Due to a defect in the freezer, the berries thawed almost completely and then froze again at some time between the eighth and twelfth month of storage. Before tasting, the frozen berries were thawed at room temperature in the original containers.

The relative palatability of the different varieties and treatments was determined by taste panels of men and women from the University of Connecticut. In the first four tests they included visitors at Farm and Home Week as well. The number of tasters in any single test varied from 12 to 53. In tests 7 to 9, inclusive, the same panel of 16 tasters served throughout.

The samples of thawed berries were presented to the tasters in simple factorial designs in the first five tests (Table 1). In the last four tests, due to the multiplicity of variables, the judges tasted and ranked the samples in balanced incomplete blocks of four, five, or six. Previous to analysis the ranks were transformed to scores for normalizing ordinal data, Fisher and Yates (1948).

RESULTS AND DISCUSSION

Relative Palatability of Eight Varieties of Fresh Blueberries

All eight varieties of the fresh blueberries were ranked in order on each of three dates (July 24, 31, and August 5) by the same 12 tasters. From their average scores, the varieties could be ranked provisionally in decreasing order of preference as follows: Jersey, Atlantic, Pioneer, Pemberton, Concord, Wareham, Rubel, and Cabot. The mean scores of the first three were nearly equal, of the next three more widely spaced, with Rubel and Cabot more divergent. The mean difference between varieties, however, was not significant when compared with the interaction of varieties x tasters ($P = 0.07$). The order of preference varied significantly, both from day to day and from taster to taster. Concord received the highest score on the first test day and Atlantic on the third test day, while on the other two days each was outranked by four or more other varieties. Of the tasters who discriminated clearly between varieties, some preferred Jersey, some Atlantic, and some Pioneer.

The palatability scores were related to certain physical characteristics of the blueberries to determine the influence of the latter upon the differences among varieties. The effect of percentage of unripe berries, of the percentage of split and overripe berries, and of berry size was measured by an analysis of covariance. None of these factors modified the day to day fluctuations in varietal preference, and the percentage of imperfect fruit prior to culling had no effect on the average scores of the eight varieties. Differences in berry size, however, accounted for two-thirds of the differences between varietal scores, the varieties having the smallest berries scoring the lowest in palatability. Hence in comparisons of fresh berries, the factor for size alone accounted for the over-all differences in varietal preference, when these were compared with the variety x date interaction. Atlantic and Jersey were the largest, Wareham and Pemberton somewhat smaller, Concord and Pioneer still smaller, and Rubel and Cabot the smallest of the berries.

The above preferences are somewhat at variance with those reported by other workers. The "dessert" quality of these varieties as listed by

Darrow, Wilcox, and Beckwith (1944) in decreasing order was Wareham, Pioneer, Atlantic, Concord, Pemberton, Rubel, Jersey, Cabot, which compared with decreasing berry size as follows: Atlantic, Pemberton, Jersey, Concord, Wareham, Pioneer, Cabot, Rubel. Jersey, especially, differed in the present study from Darrow's rating in quality although not in size. Of the comparable varieties tested by Moon, *et al.* (1936), the order of decreasing preference was Pioneer, Cabot, Concord, Rubel.

TABLE 1

Description of Palatability Tests on Frozen Blueberries

Test	Length of frozen storage (approximate)	No. of tasters	No. of samples	Varieties	Sweetening	Experimental design
1	10 days	37	6	Pioneer	None and sucrose sirup 33.3 and 37.5 pct.	Factorial
2	10 days	36	6	Concord	None and sucrose sirup 33.3 and 37.5 pct.	Factorial
3	10 days	35	6	Jersey	None and sucrose sirup 33.3 and 37.5 pct.	Factorial
4	10 days	16	6	Rubel	None and sucrose sirup 33.3 and 37.5 pct.	Factorial
5	9 months	53	6	Jersey	None, dry sucrose, and sucrose sirup 35.5 pct.	Factorial
6	10 months	15	21	Jersey Rubel Wareham Concord Pioneer Pemberton	None, dry sucrose, and sucrose sirup 35.5 pct.	Balanced incomplete blocks
7	14 months	16	16	All eight	Dry sucrose	Balanced incomplete blocks
8	15 months	16	16	Atlantic Jersey Pemberton Wareham	None[1] and sucrose sirup 35.5 pct.	Balanced incomplete blocks
9	18 months	16	13	Atlantic Concord Pioneer Rubel	None[1] and sucrose sirup 35.5 pct.	Balanced incomplete blocks

[1] Sweetened with sucrose sirup (35.5 per cent) after thawing and before tasting.

Effects of Prefreezing Treatments on the Quality of Frozen Blueberries

Varieties. Because of the large number of treatments, all eight varieties were included only in test No. 7 (Table 1). Six varieties were compared directly in test No. 6 and four varieties in Nos. 8 and 9. The results of the frozen berries have been summarized together with those on the fresh berries in Table 2. In each case the error term was based upon the interaction of varieties x tasters wherever this mean square differed appreciably from that for the remaining interactions. In order that they might be compared more readily, the average scores in the separate tests have been converted to units of the standard error of the difference between two means. Because the five experiments compared different groups of varieties the ranks in the last column have been assigned from a visual comparison of the coded scores rather than statistically. In four of the five experiments an F test for the over-all difference between varieties was statistically significant.

TABLE 2

Palatability Scores for Eight Varieties of Blueberries, Both Fresh and Frozen, in Units of the Standard Error

Varieties	For fresh berries	For frozen berries in tests No.				Varietal ranks
		6[1]	7[2]	8[2]	9[1]	
Jersey	0.90	2.19	1.55	2.19	.....	1
Pioneer	0.73	2.18	2.23	.....	1.45	1
Pemberton	0.56	2.20	0.06	—0.10	.....	2
Wareham	0.09	2.06	1.46	—1.29	.....	2
Atlantic	0.84	.....	—0.74	—0.81	1.29	3
Concord	0.23	—1.15	—0.58	.....	—1.27	4
Rubel	—0.98	1.47	—2.01	.....	—0.84	5
Cabot	—2.37	.....	—1.97	.....	.....	6
D. F. in error	14	280	105	45	45	....
Significance among varieties, P	0.07	<0.01	<0.001	<0.01	<0.025	....

[1] Based on totals for unscalded berries sweetened with sirup.
[2] Based on totals for all treatments.

Varietal preferences were affected but little by differences in treatment. Scalding and sweetening seemed to improve Wareham more than Jersey or Rubel in test six (significant interaction). This may have been related to a higher proportion of split berries in the Wareham variety and in consequence less deterioration in the treated than the untreated samples.

Sweetening. In every test the berries sweetened before freezing were preferred to those frozen unsweetened, a difference which was highly significant ($P < 0.001$) in four of the first six tests (Table 3). Since this preference might have been due to the sweetness alone, the unsweetened frozen berries in tests eight and nine were sweetened with sucrose sirup before tasting. Those sweetened before freezing were still preferred, in test eight significantly ($P = 0.01$). Dry sucrose was compared with sucrose sirup as a sweetening agent in tests five and six and received a smaller average score in both experiments. The difference was not significant in either test. In all of the above comparisons the interaction of tasters x sweetening provided the error term. In most of the tests this disagreement

among tasters was larger than the higher order interactions, and in four tests (Nos. 1, 2, 4, and 9) significantly higher. It is evident that some of them preferred the unsweetened to the sweetened berries. Kirkpatrick (1944), Woodroof and Atkinson (1944), and Moon, *et al.* (1936) likewise observed that sweetening before freezing was preferred.

Scalding. All nine tests of frozen berries compared scalded and unscalded fruit. The unscalded frozen berries were preferred to the scalded after a storage period of only 10 days (Table 4). Similarly, unscalded Jersey berries were preferred after storage for nine months. In the four tests conducted on berries stored for 10 to 18 months, the only significant preference was in favor of the scalded berries. These later results may

TABLE 3

Palatability Scores for Frozen Blueberries, Frozen Without Sweetening, With Dry Sucrose, and With Sucrose Sirup, in Units of the Standard Error

Test	Variety	No. of tasters	Degrees of freedom	Palatability scores		
				For unsweetened berries	For berries sweetened with dry sucrose	For berries sweetened with sucrose sirup
1	Pioneer	37	72	—5.36	.....	2.68[2]
2	Concord	36	70	—3.31	.....	1.65[2]
3	Jersey	35	68	—1.03	.....	0.52[2]
4	Rubel	16	30	—0.25	.....	0.12[2]
5	Jersey	53	104	—3.65	1.65	2.01
6	Jersey Rubel Wareham	15	28	—7.02	2.05	3.91
8	Atlantic Jersey Pemberton Wareham	16	15	—1.41[1]	.....	1.41
9	Atlantic Concord Pioneer	16	15	—0.70[1]	.....	1.01

[1] Sweetened with sucrose sirup shortly before tasting.
[2] Average scores for 33.3 and 37.5 per cent sucrose sirup; others all 35.5 per cent.

have been influenced by the fact that at some time between the eighth to the twelfth month of the storage period the berries had almost completely thawed and then frozen again, due to a faulty freezer. However, the quality of the berries remained surprisingly high throughout the period of the study, and only rarely was an inferior flavor noted.

In a few cases the varieties responded differently to scalding, as has been discussed previously. In only one test (No. 5) were significant differences ($P < 0.05$) due to scalding associated with the type of sweetening agent. In the unscalded berries the preferred sweetening agent was sugar sirup, while in the scalded it was the dry sugar. The difference in preferences of the tasters was significant in the tests on berries stored 10 days, some of the tasters preferring the scalded to the unscalded fruit. In test

seven, with all eight varieties frozen with dry sucrose, there was also a significant difference among tasters; in the remaining tests the tasters agreed with one another.

Woodroof and Atkinson (1944) report that scalding prevented the development of a woody texture and also improved the flavor and aroma in frozen blueberries. On the other hand, Kirkpatrick (1946) found that the unscalded berries had a fresher flavor and remained more firm, yet exhibited no woodiness. Shimer and Purinton (1948) reported that frozen berries of the lowbush type, scalded and unsweetened, developed a toughness of texture. In the present study the eight varieties, all of the highbush type, showed no evidence of woodiness in either scalded or unscalded samples.

TABLE 4

Palatability Scores for Frozen Blueberries, Frozen Unscalded and After Scalding, in Units of the Standard Error

Test	Variety	Storage period	No. of tasters	Degrees of freedom	Palatability scores		Significance between treatments
					For unscalded berries	For scalded berries	
1	Pioneer	10 days	37	36	1.53	—1.53	$P < 0.01$
2	Concord	10 days	36	35	1.60	—1.60	$P < 0.01$
3	Jersey	10 days	35	34	4.16	—4.16	$P < 0.001$
4	Rubel	10 days	16	15	2.02	—2.02	$P < 0.01$
5	Jersey	9 months	53	52	1.53	—1.53	$P < 0.01$
6	Jersey Rubel Wareham	10 months	15	70[1]	—1.75	0.58	$P < 0.05$
7	All eight	14 months	16	15	—0.81	0.81	.....
8	Atlantic Jersey Pemberton Wareham	15 months	16	15	0.12	—0.12	.....
9	Atlantic Concord Pioneer	18 months	16	15	—0.27	0.72	.....

[1] Based upon all first and second order interactions of tasters with blanching.

DISCUSSION

The choice of freezing method might be determined by the way in which the blueberries are to be used. If intended for sauce they should be sweetened with sirup or dry sugar before freezing. If they are to be made into pies they might well be frozen unsweetened and unblanched (unpublished data). Blueberry pies made from frozen, unsweetened berries (sweetened after thawing) proved as palatable as those made from frozen, sweetened berries.

Frozen berries were preferred to the canned, both as sauce and in pies.

Blueberries maintained their high quality even after long periods of frozen storage. Blueberry pies made from berries which had been held in

frozen storage for eight or nine months, and themselves frozen for over a year, were still considered of good quality.

The sweetness of the berries was greatly affected by the degree of ripeness at the time of picking. Even though the color of the unripe berries changed from red to blue rather quickly after picking, they remained sour. The best quality of frozen berries were those picked when thoroughly ripe.

SUMMARY AND CONCLUSIONS

Eight varieties of cultivated highbush blueberries have been compared as to palatability, both fresh and after freezing. The berries were frozen without sweetening, with dry sucrose, and with sucrose sirup, in each case with and without scalding. The relative palatability of the different varieties did not change significantly after freezing. Ranks have been assigned on the basis of five tests in each of which four or more of the varieties were compared. Jersey and Pioneer were preferred to the other varieties, with Pemberton and Wareham receiving slightly lower scores. Atlantic, Concord, Rubel, and Cabot followed in order. The size of the berry influenced the preference of the tasters, the varieties with the smallest berries receiving the lowest scores.

Berries sweetened before freezing were preferred to those frozen unsweetened, even when the latter were sweetened after thawing. However, pies made with frozen berries sweetened after thawing were liked fully as well as those made with the berries sweetened before freezing.

The effect of scalding varied appreciably only in relation to the length of frozen storage. In berries stored for short periods, the unscalded were preferred; after longer periods (about one year or more) scalding seemed to have little effect.

Tasters varied in their preferences of both fresh and frozen berries.

The general quality of the frozen blueberries was excellent. No woodiness was encountered and only rarely was any inferior flavor noted.

Acknowledgment

The authors acknowledge the aid of Catherine Cowell and Margaret Harvey, and all the subjects who acted as tasters throughout the experiment. They are indebted to the Department of Plant Science at the University of Connecticut for supplying the blueberries.

REFERENCES

Darrow, George M., Wilcox, R. B., and Beckwith, Charles S., 1944. Blueberry growing. U. S. Dept. Agr. Farmers' Bull. No. 1951.

Fisher, Ronald A., and Yates, Frank, 1948. Statistical Tables for Biological, Agricultural and Medical Research. Third ed. Hafner Publishing Company Inc., New York.

Kirkpatrick, Mary E., 1946. Frozen blueberries: home processing and use. J. Home Econ., **38**, 291-292.

Moon, H. H., Smart, Helen F., and Caldwell, Joseph S., 1936. Varietal suitability of cultivated highbush blueberries for freezing in consumer packages. Fruit Products J., **15**, 229-231, 248, 251.

Shimer, S. R., and Purinton, H. J., 1948. The ascorbic acid and carotene content of fresh and frozen New Hampshire berries. New Hampshire Agr. Exp. Sta. Tech. Bull. 92.

Woodroof, J. G., and Atkinson, Ida S., 1944. Blueberries for freezing improved by blanching. Refrig. Eng., **48**, 275-278, 314.

NUTRITIONAL VALUES OF PROTEINS IN VARIOUS CEREAL BREAKFAST FOODS[1]

BARNETT SURE
Department of Agricultural Chemistry, University of Arkansas, Fayetteville, Arkansas

[Received for publication, December 15, 1950]

It has been known for many years that heat processing can impair the nutritive value of proteins, McCollum and Davis (1915). The sensitivity of cereal proteins to heat has been known since the experiments of Morgan (1931). In 1943 information became available on the influence of extreme heat, applied for a short time, on the biological value of the proteins in cereal breakfast foods. Stewart, Hensley, and Peters (1943) have shown in feeding experiments on growing rats that the ratio of gain in body weight to protein consumed, when the food intakes of the animals are equalized, is depressed by two-thirds by the gun explosion of an oats-corn-rye mixture, and by three-fourths by the gun explosion of oats alone. It would appear, then, that such methods of preparation will yield breakfast cereals whose protein value in nutrition bears little relation to that of the raw cereals from which they were derived. In this connection, Mitchell, Hamilton, and Beadles (1945) state: "In the public interest, it would seem to be desirable to process cereals for human consumption by methods that are less destructive of nutrient content."

Patton (1950) has recently reviewed the literature on the heat-processing damage to protein foods, and has pointed out that under conditions of dry heat and absence of carbohydrate the injury is due to enzymic reactions; e.g., the processing of proteins produces a new lysine linkage, which is either not digestible by enzymes or is so slowly digested that part of the lysine enters the blood stream too late to participate with the rest of the assimilated amino acids in tissue formation. Under conditions of moist heat and the presence of reducing substances, true amino acids may be largely responsible for protein injuries in processed foods. Since most breakfast cereals are processed foods, it was thought of interest to investigate the protein values of various cereal breakfast foods.

Breakfast foods vary considerably in protein, vitamin, and mineral content and, therefore, would of course vary markedly in their nutritional values. Unquestionably, all cereal breakfast foods are good sources of calories. When a breakfast is composed of 1 ounce of cereal, 4 ounces of milk, and 1 teaspoonful sugar, 104 of the 202 calories are furnished by the cereal, Reasor (1946). However, information is lacking on how the various processed cereal breakfast foods rate in the biological value of their proteins without consideration to the supplementary value of the milk added. Oatmeal and Cream of Wheat are often cooked with water

[1] With the technical assistance of Leslie Easterling, Joy Dowell, and Mary Crudup. Research paper No. 941, Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

instead of milk. In order to supply such information a study was initiated, the results of which are summarized in Tables 1 and 2.

TABLE 1

Total Protein Content of Various Breakfast Foods

Breakfast food	Total protein
	pct.
Corn Flakes	6.2
Corn-Soya	18.0
Kix	7.8
Wheaties	9.6
Puffed Wheat	13.2
Cream of Wheat	11.2
Grape Nuts	9.2
Grape Nut Flakes	10.1
40 Per Cent Bran Flakes	9.5
Quick Oats	17.0
Cheerios	14.9
Instant Ralston	13.8
Pablum	14.6 to 15.1
Cerevim	17.9 to 19.2

EXPERIMENTAL PROCEDURE

This investigation was carried out on Wistar strain, albino rats. The animals were about four weeks old when started on the experiments and weighed 50 to 53 gm. each. They were weighed weekly, and records were kept of the food consumption. The experimental period for all the groups of animals was ten weeks with the exception of those on Corn Flakes, Kix, and one group on Corn-Soya (Table 2) which were fed for only six weeks. Growth on the proteins in Corn Flakes and Kix were so poor that no animals survived over six weeks on rations which provided the proteins from such sources; therefore, the experimental period had to be reduced to six weeks. One experiment was conducted on Corn-Soya for six weeks, so as to have a basis for comparison with the one on Corn Flakes; and a duplicate experiment on Corn-Soya was conducted for ten weeks.

The rations contained 2 per cent Cellu-flour for roughage, 4 per cent of Sure's salt mixture No. 1 (1941), 8 per cent vegetable shortening, 2 per cent cod liver oil, and 1 per cent wheat germ oil. The breakfast foods furnished the only source of proteins in the rations which were fed, depending on their total nitrogen content, at protein levels of 5, 8, and 10 per cent. Because of their low nitrogen content Corn Flakes and Kix could be fed only at a 5 per cent plane of protein intake.

The following crystalline components of the vitamin B complex were administered daily to each animal separately from the ration: 25 μg. each of thiamine, riboflavin, pyridoxine, and niacin, 150 μg. calcium pantothenate, 3 mg. p-aminobenzoic acid, 6 mg. choline chloride, and 1 mg. inositol.

From the gains in body weight per gram of protein intake, the protein efficiency ratios (PER) were calculated. The protein content of the breakfast foods was determined by analyzing the total nitrogen and multiplying same by 6.25. These results are given in Table 1. For breakfast foods made from wheat, the factor 5.7 was used, as recommended by Jones (1931).

RESULTS

From Table 2 it is evident that Corn Flakes, 40 Per Cent Bran Flakes, Kix, Grape Nuts, Grape Nut Flakes, and Puffed Wheat all fall in the same category. The proteins in none of these breakfast foods have permitted any gains of body weight. Only losses of body weight were encountered and a great proportion of the animals on these breakfast foods died be-

TABLE 2

Relative Nutritional Values of Proteins in Various Cereal Breakfast Foods
(Average results per animal)

Cereal breakfast food	Number of animals	Protein in ration	Changes in body weight	Mortality	Total food intake	Protein intake	PER[1]
		pct.	*gm.*	*pct.*	*gm.*	*gm.*	
Corn Flakes[2]	9	5	−8.5	11.1	159.3	8.0	−1.06±0.16[3]
Corn Soya[2]	9	5	+13.6	.....	309.4	15.5	0.88±0.14
Corn Soya	12	5	+15.2	.....	505.5	25.3	0.60±0.15
	12	8	+51.3	.....	569.0	45.5	1.13±0.07
	12	10	+78.7	.....	685.0	68.5	1.15±0.04
Kix[2]	12	5	−6.7	65.0	149.1	7.5	−0.89±0.10
Wheaties	12	5	−1.6	.....	375.1	18.8	−0.85±0.05
	12	8	+4.4	.....	352.9	28.2	0.16±0.02
Puffed Wheat	12	10	−10.2	.....	261.3	26.1	−2.50±0.40
Cream of Wheat	12	5	+12.2	.....	400.0	20.0	0.61±0.07
	12	8	+32.5	.....	451.3	36.0	0.90±0.07
	12	10	+67.3	.....	561.9	56.2	1.20±0.16
Grape Nuts	12	5	−11.4	65.0	239.4	12.0	−0.95±0.03
	12	8	−7.0	25.0	294.9	25.5	−0.28±0.01
Grape Nut Flakes	12	5	−11.4	50.0	309.3	15.5	−0.73±0.02
	12	8	−6.8	75.0	275.6	22.0	−0.31±0.01
40% Bran Flakes	12	5	−6.3	75.0	325.3	16.3	−0.39±0.06
	12	8	−5.8	50.0	300.0	24.0	−0.24±0.04
	12	10	−2.3	8.3	336.1	33.6	−0.07±0.01
Quick Oats	12	5	+49.1	.....	594.4	29.2	1.67±0.03
	11	8	+91.8	.....	659.8	52.7	1.74±0.04
	11	10	+111.3	.....	679.2	67.9	1.64±0.06
Cheerios	12	10	+66.6	.....	591.1	59.1	1.13±0.04
Instant Ralston	12	5	+34.2	.....	521.6	26.1	1.31±0.14
	12	8	+83.4	.....	722.2	57.8	1.45±0.05
	12	10	+124.2	.....	825.6	82.5	1.51±0.05
Pablum	12	5	+21.3	.....	452.0	22.6	0.94±0.05
	12	8	+60.0	.....	596.7	47.7	1.26±0.04
	12	10	+93.3	.....	730.5	73.0	1.28±0.04
Cerevim	12	5	+36.7	.....	590.0	29.5	1.24±0.25
	12	8	+82.9	.....	725.0	58.0	1.42±0.02
	12	10	+116.7	.....	793.3	79.3	1.47±0.02

[1] PER signifies protein efficiency ratio, expressed as gains in body weight per gram of protein intake. The negative sign preceding PER signifies the loss of weight per gram of protein intake.
[2] Standard deviation of the means.
[3] These groups of animals were 6 weeks on experiments. All the rest of the groups were 10 weeks on experiments.

tween the second and ninth weeks. Of these, Corn Flakes showed a slightly better source of proteins, as judged by the low incidence of mortality. In contrast, it was previously demonstrated that when wheat was fed as milled enriched flour and when corn was fed as degerminated white corn meal to albino rats at 5 per cent protein levels, growth took place at a slow rate but without any accompanying mortality, Sure (1947).

On a 5 per cent level of protein, Wheaties are a poor source of proteins. On an 8 per cent protein intake, while allowing small increments of growth, Wheaties are still a very poor source of proteins. Even on a 10 per cent protein level, Puffed Wheat proved a very poor source of proteins whereas milled wheat fed as enriched flour on the same level of protein permitted appreciable growth, Sure (1946). The proteins in Cream of Wheat compare favorably in protein efficiency with those obtained by feeding wheat proteins in enriched wheat flour, Sure (1946). However, when consumed with milk, as they generally are, the heat damage to these cereal proteins may be adequately corrected by the milk proteins with which they are combined.

Corn Flakes have a low protein content of 6.2 per cent and are an unsatisfactory source of protein biologically. However, when corn is mixed with soybeans as in Corn-Soya, the protein content is 18.0 per cent and is much improved from the nutritional standpoint (Table 2).

Cheerios, made from oat flour, showed a value at a 10 per cent protein level (the only one fed) similar to that of Cream of Wheat. Under the conditions of the present experiment, Quick Oats, Instant Ralston, Cerevim, and Pablum proved to be the best of the breakfast foods tested from the standpoint of protein efficiency.

SUMMARY

A biological study was made of the nutritional values of proteins in various cereal breakfast foods, using the Wistar strain albino rat as the experimental animal. The yardsticks for evaluation employed were gains in body weight per gram of protein intake, which is an index of the protein efficiency ratio.

When tested under the conditions of the present experiment, the proteins in Kix, Grape Nuts, Grape Nut Flakes, Puffed Wheat, 40 Per Cent Bran Flakes, Corn Flakes, and Wheaties were poor sources of proteins, as evidenced from losses of body weights or inadequate gains in body weight of the rats during the experimental periods. Corn-Soya, Cream of Wheat, and Cheerios proved to be fairly good sources of proteins for growth. The best results, from the standpoint of protein efficiency, were obtained with Quick Oats, Cerevim, Pablum, and Instant Ralston.

Acknowledgment

We wish to express our appreciation to the M & R Dietetic Laboratories for their generous contribution of Cerevim; to the Mead, Johnson & Company for their liberal supply of Pablum.

REFERENCES

JONES, D. B., 1931. Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. U.S.D.A. Circular No. 183, 1-21.

MCCOLLUM, E. V., AND DAVIS, M., 1915. The cause of the loss of nutritive efficiency of milk. J. Biol. Chem., **23**, 247-254.

MITCHELL, H. H., HAMILTON, T. S., AND BEADLES, J. R., 1945. The importance of commercial processing for the protein value of food products. J. Nutrition, **29**, 13-25.

MORGAN, A. F., 1931. The effect of heat on the biological value of cereal proteins and casein. J. Biol. Chem., **90**, 771.

PATTON, A. R., 1950. Present status of heat-processing damage to protein foods. Nutrition Revs., **8**, 193-196.

REASOR, A. J., 1946. A cereal source book, Cereal Institute, p. 12.

STEWART, R. A., HENSLEY, G. W., AND PETERS, F. N., 1943. The nutritive value of protein. I. The effect of processing oat protein. J. Nutrition, **26**, 519.

SURE, B., 1941. The existence of a new dietary factor essential for lactation. J. Nutrition, **22**, 499-514.

———, 1946. Nutritional improvement of cereal flours and cereal grains. J. Am. Dietet. Assoc., **22**, 494-502.

———, 1947. Further studies on nutritional improvement of cereal flours and cereal grains with yeast. J. Am. Dietet. Assoc., **23**, 113-119.

EFFECT OF CRYSTAL SIZE ON SALT PENETRATION OF PORK LOINS[1]

F. H. SMITH, T. N. BLUMER, ALEXA C. WILLIAMS, AND W. E. TYLER

North Carolina State College of Agriculture and Engineering, Raleigh, North Carolina

[Received for publication, December 22, 1950]

This investigation was undertaken because it has been reported by frozen food-locker operators and others that the rate of salt penetration into cuts of meat varied with the size of the salt crystals.

The Bureau of Animal Industry (1941) reported that without a knowledge of distribution of salt in muscle tissue the quantity of salt may be of little value. Therefore, the depth of penetration and the concentration at any particular depth in the loins were determined in this study.

METHODS

Six loins from six different hogs of similar weight and breeding were selected for this study. An identical portion of each loin was used in order to reduce the variation to a minimum.

The portion of each loin used was obtained by cutting the loin through the second thoracic vertebra (break in the back) and extending anteriorly for twelve inches. This twelve-inch portion of the loin was boned, trimmed free of fat, and separated into three pieces, four inches in length.

Each four-inch piece of loin was enclosed in aluminum foil with the exception of the end to which the salt was applied. A cut was made around the loin a quarter inch from the end of each sample. The aluminum foil was then inserted into the cut in the loin and secured with string in such a way that the drip or run-off would flow down over the aluminum foil without touching the sample. The aluminum foil extended well beyond the opposite end of the loin. This supported it in a beaker above the level of the run-off liquid. The salt was applied to the exposed end of the loin.

The electrometric titration method was used for determining salt as described by Brady, Smith, Tucker, and Blumer (1949).

EXPERIMENTAL PROCEDURE

Fine flake, medium flake, and granulated salt were used in this study. Salt of one of these crystal structures was applied to each of the loin samples and after a given time, the depth of penetration and the amount of salt absorbed were determined chemically.

Each of six loins was divided into three samples for this experiment. All three samples taken from each loin were randomized for type of salt treatment so that 12 grams of one type of salt were applied evenly over the exposed end of the sample randomly assigned to that salt treatment.

The samples were stored at 4.5°C.(40°F.) for a period of eight days, then were removed and sliced into cross sections one-half cm. thick. Alternate cross sections were analyzed because a preliminary study showed this to be sufficient for chemical analysis.

The average percentage of salt for the six loin replications of each salt treatment in positions one through 13, as indicated, is shown in Table 1.

---

[1] Contribution from the Department of Animal Industry, North Carolina Agricultural Experiment Station. Published with the approval of the Director as Paper No. 371 of the Journal Series. The authors wish to acknowledge their indebtedness to Dr. H. L. Lucas for the statistical analysis of the data.

It may be observed that these are only very slight differences in concentration of the three types of salt at any depth of penetration.

Statistical analysis of the analytical data showed that there were no significant differences between the three types of salt. The salt penetration was not influenced by the fat content which ranged from 1.78 to 13.14 per cent.

TABLE 1

Average Percentage of Salt in Loin Cross Section [1]

Cross section No.	Depth in cm.	Fine flake	Medium flake	Granular
		pct.	pct.	pct.
1	0-0.5	12.80	13.21	13.63
3	1-1.5	7.23	7.77	8.30
5	2-2.5	3.24	3.97	4.29
7	3-3.5	1.40	1.83	2.11
9	4-4.5	0.56	0.82	0.99
11	5-5.5	0.30	0.40	0.54
13	6-6.5	0.24	0.24	0.26

[1] Penetration of salt measured after eight days in storage at 4.5°C.(40°F.).

This study was repeated to determine the amount of the different types of salt in the residue not absorbed and the amount of salt in the drip collected from each loin sample. Weights from six replications of fine flake, medium flake, and granulated salt in the residue were, respectively, 3.58, 3.83, and 3.07 gm.; and in the drip, 1.01, 1.15, and 1.48 gm. These values deducted from the amount of salt applied shows that an average of 7.41, 7.02 and 7.45 gm. of fine flake, medium flake, and granulated salt was absorbed respectively. There were no statistical differences in these amounts.

Hams cured with these three types of salt gave results which were essentially the same as those obtained with the loins.

DISCUSSION AND CONCLUSION

This study showed no appreciable difference in the quantity or rate of penetration of the three types of salt into pork loin muscle. Similar results were obtained on hams and are to be expected with other pork cuts. The extra cost of using flake salt can not be justified on the basis of the amount of salt absorbed or the rate of penetration. However, it may have some advantages over granulated salt in ease of application.

These data show that the salt penetrated the loins at the rate of approximately 0.5 cm. per day. There were no significant differences in the penetration rate of the three types of salt.

The possible reason for the apparent visible difference in rate of salt absorption reported may be due to the difference in weight per unit volume. Fine flake salt weighs approximately 83.83 per cent as much as an equal volume of granulated salt, while medium flake salt weighs 77.44 per cent as much as an equal volume of granulated salt. In instances where the amount of flake salt for curing meat is measured rather than weighed, the actual quantity of salt used is less than an equal volume of granulated salt. This may account for the belief that flake salt is absorbed more rapidly than granulated salt.

SUMMARY

No difference was noted in the rate of penetration or the quantity of salt absorbed when comparing fine flake, medium flake, and granulated salt in pork loins.

There were no significant differences in the moisture loss or in the quantity of salt in the drip, or liquid run-off, for the treatments used.

REFERENCES

U. S. Department of Agriculture, Bureau of Animal Industry, 1941. Report of the Chief of the Bureau of Animal Industry, page 17.

BRADY, D. E., SMITH, F. H., TUCKER, L. N., AND BLUMER, T. N., 1949. Characteristics of country style hams as related to the sugar content of the curing mixture. Food Research, **14**, 303-309.

ANNOUNCEMENT OF SAMUEL CATE PRESCOTT AWARD COMPETITION FOR STUDENT MEMBERS OF THE INSTITUTE OF FOOD TECHNOLOGISTS

An award of $100.00, designated as the SAMUEL CATE PRESCOTT AWARD, is to be made by the Northeast Section of the Institute of Food Technologists to the student presenting for this competition the best written account of a research project in the field of food technology.

Those eligible for the competition include all student members of the Institute of Food Technologists in good standing or persons who were formerly student members but who attained their bachelors' degree no more than 12 months previously.

To be included in the present year, qualified competitors are required to submit their written reports in this competition to the Institute of Food Technologists, 176 West Adams St., Chicago 3, Illinois, previous to August 25, 1951.

DIFCO

Culture Media for Examination of

MILK and DAIRY PRODUCTS

for Plate Counts

BACTO-TRYPTONE GLUCOSE EXTRACT AGAR is recommended for routine plate counts of bacteria in milk. This medium conforms to all requirements of "Standard Methods for the Examination of Dairy Products" of the American Public Health Association, except that it does not contain skim milk.

BACTO-PROTEOSE TRYPTONE AGAR is recommended for determinations of the total bacterial plate count of certified milk. This medium is prepared according to the specifications of "Methods and Standards for Certified Milk" of the American Association of Medical Milk Commissions.

for Detection of Coliform Bacteria

BACTO-VIOLET RED BILE AGAR is widely used for direct plate counts of coliform bacteria. Upon plates of this medium accurate counts of these organisms are readily obtained.

BACTO-BRILLIANT GREEN BILE 2%

BACTO-FORMATE RICINOLEATE BROTH are very useful liquid media for detection of coliform bacteria in milk. Use of these media is approved in "Standard Methods."

for Detection of Molds

BACTO-POTATO DEXTROSE AGAR is an excellent medium for detection and enumeration of molds and yeasts in butter and other dairy products. The formula of this medium corresponds exactly with that specified in "Standard Methods."

BACTO-MALT AGAR is also widely used for determinations of the mold and yeast count of dairy products and for control of the sanitary conditions of manufacture.

for Cultivation of Lactobacilli

BACTO-TOMATO JUICE AGAR

BACTO-TRYPSIN DIGEST AGAR support luxuriant and characteristic growth of *Lactobacillus acidophilus*, and are well adapted for use in establishing the number of viable organisms in acidophilus products. These media are also widely used for estimation of the degree of implantation of *L. acidophilus*.

Specify "DIFCO"

The Trade Name of the Pioneers in the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES

DETROIT 1, MICHIGAN